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INTRODUCTION

In order to provide for the printing of scientific papers, which formerly constituted a large part of the volume known as the Annual Report, the Board of Trustees has authorized a new journal, to be known as the ANNALS OF THE MISSOURI BOTANICAL GARDEN. The Annals will appear four times a year, in March, May, September, and November, and contain only scientific contributions from members of the staff of the Garden, from the faculty and graduate students of the Henry Shaw School of Botany of Washington University, and from visiting botanists doing all or a part of their work at the Garden. The increase in original contributions available for publication, due to the additions to the staff and the greater number of graduate students, makes it no longer possible to follow the practice of the past and print papers from sources other than the Garden.

The publication of a monthly bulletin by the Missouri Botanical Garden, in which appear promptly the annual reports of the officers of the Board, and of the Director, together with popular accounts of the various activities of the Garden; and the provision for the printing of scientific papers in the Annals, has made it advisable to discontinue the Annual Report, which was published each year from 1890 to 1912. The Twenty-third Annual Report, therefore, marks the close of this series.

The Annals will be maintained upon a strict subscription basis, using it in exchange only when its equivalent can be obtained. Some of the institutions and societies, the publications of which have been received in exchange for the Annual

Report, issue nothing of value to a botanical library and apparently are not interested in botany or related sciences. These have been stricken from the exchange list. On the other hand, the receipt of this number of the Annals is an indication that the Missouri Botanical Garden desires to continue the old exchange arrangement, the new journal being sent four times a year in place of the old Annual Report. Additional exchanges with publishers of journals dealing directly with botany are desired. Upon request, the monthly BULLETIN will be substituted for the Annals as an exchange with those desiring a more popular and general account of the work and scope of the Missouri Botanical Garden.

GEORGE T. MOORE
Director.

THE EFFECT OF SURFACE FILMS AND DUSTS ON THE RATE OF TRANSPIRATION

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The fungicides commonly employed are either in the form of solutions (e. g., ammoniacal copper carbonate), suspensions (lime wash and Bordeaux mixture), and powders (sulphur). The use of spray mixtures or other fungicides has become world wide, and many problems of physiological interest have arisen respecting the effects of these substances on the plants which they are designed to protect. Bordeaux mixture has been under continuous observation for a period of about twenty years, and has proved interesting in both its toxic and other relations. The striking influence of this fungicide upon sound plants has awakened widespread interest, and numerous experiments have been made to determine the nature of the effects. Bordeaux mixture consists essentially of suspension films of copper hydroxid and certain other complex (mostly hydrated), largely insoluble, copper compounds; and when properly sprayed upon plant surfaces from the best nozzles, the particles are of extreme fineness, and there is realized an almost perfect surface film. In spite of the greatest care in preparation and application, it is injurious to certain plants, such as the peach and the plum, and may not be used satisfactorily in such cases for disease control. In recent years it has been shown that the extent of the injury to the apple and other plants may be considerable, and Bordeaux mixture is in such cases being supplanted. In this discussion, however, we may omit any detailed consideration of the toxic effects of this mixture, a phase of the subject which has received much consideration in this country from Bain (2), Crandall (6), Clark

(4), Swingle (21), and others. Moreover, with the exception of incidental references, we wish to deal at this time only with its physiological action in prolonging the vitality of leaves and plants.

During the first years of the use of this spray mixture it was natural that any increased vitality of the sprayed plants would be attributed merely to the action of the fungicide in restraining fungous or insect pests. Indeed, we find no authentic suggestion of any other effect than that mentioned for eight or ten years after the discovery of this fungicide. Since 1892 there have been frequent observations indicating beyond any reasonable doubt that in the absence of all disease-producing organisms there is often prolonged vitality of the sprayed plants as contrasted with the unsprayed. The increased longevity is particularly noticeable in plants like the potato, in which, under normal conditions, the foliage frequently dies in advance of the first killing frost. Nevertheless, lengthened life in leaves of deciduous trees, notably of the apple, has likewise been reported. It is not always possible to state definitely to just what extent any apparent increased vitality is to be attributed to the physiological action of the fungicide rather than to the control of pests, and it must be said that the frequency of the phenomenon and the reliability of the observers alone preclude the possibility of constant errors in this matter.

In practical field experimentation the most significant differences in yield and vitality as a result of spraying with Bordeaux mixture have been evident in the case of the potato, and with this crop it is a matter of common observation both in Europe and America. In recent years the consecutive reports on potato spraying by F. C. Stewart (19) and his associates at Geneva, New York, suggest in a decisive way the probable magnitude of the Bordeaux influence when disease is a minor factor. In general, observers are perhaps liberal in their estimates of the gain from fungous suppression.

It will be pertinent to note a few observations and comments from the reports of the work done at Geneva. In 1904 the increase in yield from spraying potatoes five times was 233 bushels per acre. "Spraying prolonged the life of the plants 25 days. Late blight was the only trouble." In his experiments

of 1906 Stewart notes an increase in yield of 63 bushels per acre due to spraying five times. He remarks: "Late blight, early blight, flea beetles and tip burn were all factors in this experiment, but none of them caused much damage." More striking were the results the following year when an increase in yield of 73¾ bushels per acre was obtained from spraying five times. In this case it is reported: "Late blight and rot were wholly absent and early blight appeared only in traces. There was some tip burn and a light attack of flea beetles. Considering the seemingly small amount of damage done by blight and insects, it is remarkable that spraying should have increased the yield so much." In 1909 the increase in yield in spraying six times was 49¾ bushels per acre, and the comment upon this result is as follows: "Early blight, late blight and rot were all absent. Some injury from flea beetles was noticeable throughout the season. After September 1 there was considerable tip burn. As late as September 24 the difference between sprayed and unsprayed rows appeared slight. The sprayed rows held most of their foliage until killed by frost on October 14."

The senior author of this report visited the experimental plats which afforded these data in late September, 1911, prior to the killing frosts of October 27, and the contrast between the sprayed and unsprayed rows was pronounced; at the same time there was very little evidence of any disease on the unsprayed plats. Regarding the condition of the plants Stewart says: "There was no late blight whatever, only a very little early blight, and very little flea beetle injury. The unsprayed rows were affected by no disease of any consequence except tip burn, and even of that there was only a moderate amount. As the plants were still partially alive twenty weeks after planting it is clear that they could not have been very much injured by anything. Yet spraying increased the yield at the rate of 93 bushels per acre. Plainly we have here a striking example of the beneficial influence of Bordeaux in the absence of diseases and insect enemies."

Examining the comments of these and of other investigators regarding increased vitality as a result of spraying with Bordeaux, we find that where the condition of the plant is well

defined at the close of the season, or at the time of the first killing frost, the sprayed plants are almost invariably more vigorous. Often, in the practical absence of any disease, sprayed plants may remain healthy until killed by frost, while unsprayed plants may have died from a few days to a few weeks in advance of frost.

Following a recital of notable increases of yield in Connecticut as a result of spraying potatoes with Bordeaux, Clinton (5) expresses the conviction that an explanation must be found in the conservation of water. His statement follows:

"The question naturally comes up, why did the sprayed potatoes give this increased yield over the unsprayed if there was no particular injury caused by the late blight fungus? Some little benefit was no doubt derived from the prevention of the early blight, but this must have been scarcely appreciable because this fungus was not at all conspicuous these years. Again, some very small benefit may have been due to lessening insect attack, since potatoes sprayed with both Bordeaux and Paris green keep off the insects somewhat better than where sprayed only with Paris green. This is especially true as regards the potato flea beetle. But here again the gain was of a very minor kind. Ordinarily botanists have explained this increase as due to some stimulative effect the Bordeaux mixture has on the chlorophyll of the potato leaves in increasing starch production. Personally, the writer believes that the results are largely due to *conservation of moisture in the leaves in dry seasons by clogging up the stomata and water pores with the sediment of the spray.* The reasons for this belief are (1) that the potato leaves, through their numerous stomata and terminal water pores, lose water very easily, and are especially susceptible to what is known as tip burn in dry seasons; (2) that the unsprayed vines uniformly suffered earlier and more severely from tip burn than the sprayed, which were green for about two weeks after the unsprayed were dead; (3) that in 1910, which was a season like the preceding years, except with a little injury from blight at the very end of the season, spraying with 'Sulphocide' and commercial lime-sulphur, sprays with comparatively little sediment, did not prolong the life of the

vines or give increased yield, while spraying with Bordeaux mixture did."

Although this theoretical explanation did not come to our attention until the experimental work reported in this paper was complete, it was, in modified form, the only possible opinion which we felt inclined to advocate, as a clue to the increased longevity caused by Bordeaux, until the contrary evidence yielded by our experiments.

Review of Literature

The experimental work undertaken in the past to determine the nature of the Bordeaux influence (apart from direct injury) has touched mainly upon (1) questions of increased photosynthesis due either to "stimulation" of chloroplastid or chlorophyll development, or to a direct influence upon light quality; (2) changes in the respiratory rate, or surmised effects upon metabolism; and (3) a modification of the normal rate of transpiration. A few observations from the extensive literature with particular reference to its bearing on transpiration may be cited.

Rumm (16) finds that in sprayed grapes the chlorophyll content of the leaves increases and the fruit ripens earlier with a higher sugar percentage. He attributed these phenomena to the higher "assimilatory activity," and in turn relates this to the following observation on transpiration:—that abscised, sprayed twigs remain fresh longer than those unsprayed, from which it is deduced that there is a falling off in transpiration as a result of spraying. Through independent observations made during the same year, Müller-Thurgau (15) and Bayer (3) subscribe to the view that lessened transpiration follows spraying. Moreover, this confirmation of Rumm is obtained by the former through an experiment which also proclaims that the reduction in transpiration as a result of spraying may be as much as forty per cent. Nevertheless, the report referred to is extremely brief and does not indicate clearly the condition of the plants during the period of observation, a matter most important in the final interpretation of the data afforded.

Frank and Krüger (9, 10) reported some rather extensive

quantitative experiments as a result of which they conclude, contrary to Rumm, that transpiration is accelerated by spraying. They state that sprayed leaves are in general more robust, thicker and stiffer. They also report an increased yield in pot experiments from spraying. All these indications, as well as those of Leydheker (13) and others (1, 12) denote differences of yield which are so slight as to be of no fundamental importance in the present consideration. Nevertheless, the transpiration data of Frank and Krüger, as already observed, were obtained by satisfactory methods, and these are of greater interest when taken in conjunction with those of Zucker (22) who confirms their results entirely.

Schander (18) in an extensive paper reports a comparatively small amount of experimental work on transpiration, but in the cases given his results indicate a retardation of water loss after spraying. His experiments with cobalt paper were inconsistent, and twigs of *Taxus baccata* and potted bean plants were then employed, yielding the positive results noted. However, his work embraced very few plants, and the transpiration differences observed are inconsiderable. He suggests that lessened transpiration of sprayed plants is to be expected, since the Bordeaux mixture must exert a shading influence as a result of the exclusion from the leaf of certain injurious rays. He attempts to verify this assumption of partial shading by a study of leaf temperatures, but the experiments in this direction give no positive evidence for his theory. No adequate mention is made of the conditions surrounding these experiments, nor of the precautions observed.

Ewert's (8) experiments tend to substantiate the views of Rumm and Schander; but, unfortunately, the results are not satisfactory for accurate quantitative purposes, since evaporation from the pots was merely checked and not prevented,batting being employed to cover the soil surfaces. His experiments are of particular interest, however, with respect to his graph for comparative respiration in sprayed and unsprayed plants. In the sprayed plants, respiration was found to be distinctly lower than in the unsprayed. It will be noted, however, that this diminished respiration is scarcely in keeping

with the observation of Rumm and others regarding the higher assimilatory activity in sprayed plants.

It is unnecessary in this report to review the considerable literature which has accumulated bearing on the question of increased starch formation as a result of the application of Bordeaux mixture, especially as it is proposed to discuss this phase of the subject in a later paper.

METHODS

As indicated in the title, the experimental work here reported is concerned merely with the transpiration of sprayed and unsprayed leaves or plants. Other effects of sprays and dusts may be communicated in subsequent reports. In general, the methods involved are modifications of customary practices.

The methods used were of two types, the experiments being carried out either by means of (1) leaves in burette potometers connected with side arm flasks, or (2) potted tomato plants.

Potometer Experiments.—After much preliminary experimentation with a view to determining suitable leaves or twigs for potometer work, leaves of the castor bean were selected. Some of the preliminary experiments with other leaves are of interest, however, and will be referred to subsequently. Castor bean (*Ricinus communis*) leaves offer some special advantages, especially (1) large surfaces, (2) resistance towards Bordeaux mixture, and (3) prolonged vitality after abscision.

The burettes were connected with the side arm flasks, as indicated in plate 1, and the flasks completely filled with water. The petioles of the leaves were cemented into the mouths of the flasks by means of "plastolina." If a ring of this plastic substance is placed around the mouth of the flask when the glass is dry and a ball of the same material, larger than the mouth of the flask, is carefully attached around the petiole, then the petiole and plastolina may be plunged into the mouth of the flask and the two masses unite in a manner such as to give a perfectly air-proof, water-tight connection. It has been found desirable, for purposes of safety, to put on a second layer of the plastolina as soon as it is evident that the first permits no leakage. Even with these precautions, considerable diurnal changes in temperature may cause leakage, and it is particu-

larly important that each experiment should be carefully examined prior to making all readings. The water columns in the burettes were so gauged as to eliminate the possibility of forcing water into the leaves. The burettes were employed solely in order to get accurate readings of the water loss from hour to hour without shifting or disturbing the plants by weighing; also rapidly to get data, should it seem necessary, under changing conditions. All of these considerations proved very important, as it was found that a slight shifting of the position of the leaf affected materially the transpiration magnitudes.

For each leaf used it was necessary to get its rate of transpiration in terms of some standard in order that the ratios might be established between certain leaves prior to the addition of the spray to some of them and the ratio between the same leaves after the application. At one time it seemed possible that the revolving table method of standardizing porous cups might be applicable, but on further consideration it was believed that the use of this method in the laboratory, and the subsequent disposition of the plants in the open, would lead to errors of considerable magnitude. For our purpose it was not considered desirable to conduct the whole experiment on the revolving table, but this method will be employed in connection with our further studies. It was found very important to standardize the leaf in a given position and then permit it to remain in that position, as far as possible, throughout the experiment. This method was necessary largely because of the fact that it seemed wise to conduct the experiment in the open, during a considerable interval, at least. Further reference to the arrangement of the plants will be made in the discussion of the experimental work.

Experiments with potted plants.—For the experiments with potted plants tomatoes were used. The pots were dipped in paraffin wax and the same sealing mixture was coated over the surface of the soil. In all the experiments reported there was no leakage in any case from improper sealing. Water was added daily, or twice a day, to supply the loss by transpiration, the addition of water being made by means of a thistle tube fixed in each pot. The bell of the thistle tube was covered with paraffined paper during the entire interval. It was also

found necessary to insert in each pot a small bent tube in order to provide for the changes in air pressure.

The pots were weighed at the beginning and at the close of the experiment, but the condition of the plant and the amount of water entering readily from the thistle tube were found adequate to indicate the daily water requirements. To the total provisional transpiration quantities obtained from a summation of the quantities daily added, the differences in weight between the beginning and the close of the experiment were added or subtracted as required. From five to ten plants were employed with each kind of spray or dust used, and the plants of each lot were so distributed in the greenhouse that an equal number—so far as possible—from every group was subject to exactly the same influences. Moreover, positions in the greenhouse were shifted several times during the observation intervals of from ten days to two weeks. As a result of a large amount of experimental work in the greenhouse it has become apparent that the points just referred to are important. Plants situated nearer the edges of the benches, or those which receive drafts from opening doors or from convection currents, show considerable differences in transpiration rates, and this should be obviated.

The leaves in the potometer work and the potted plants were sprayed or dusted liberally, and in the case of the sprays, in particular, care was taken to cover completely with a fine spray of the material both surfaces of the leaves. The dust applications were made in the late afternoon when the leaf surfaces were less dry, and after dusting the upper surfaces of the leaves the plants were inverted and the lower surfaces equally well treated. The dusts were prepared by grinding to an impalpable powder in a mortar.

The Bordeaux mixture employed was made by the 4-6-50 formula, the weights of ingredients for making small quantities being approximately as follows:

$CuSO_4$	9.6 grams
CaO	14.4 grams
Water	1000 cc.

The weak Bordeaux was one-half the strength of the above. The $Ca(OH)_2$ was prepared by slaking gradually 60 grams of

CaO in 1 liter of water; and the mixture designated $Al(OH)_3$ was prepared by mixing two solutions each of 900 cc., the one containing 26 grams of $AlCl_3$ and the other 30 grams of CaO (slaked as for the Bordeaux mixture). The clay suspension consisted of 90 grams of fine air-dried clay in 1 liter of water. The lime-sulphur employed was the usual 1–25 strength.

EXPERIMENTAL DATA AND DISCUSSION

It will be observed from the brief review of earlier work that the evidence regarding the effect of Bordeaux mixture on the transpiration rate is inconsistent. A majority of the observers adopt the view that the effect of this surface film is to reduce the transpiration. On a priori grounds this view would seem to be logical, since it would indicate a water conservation to which, in dry seasons at least, the plant might respond with increased vitality and yield. Nevertheless, it was believed that the experimental evidence at hand was of insufficient scope to establish this view of it. Contrary to expectations, all of our more important experimental evidence and observations are antagonistic to the a priori assumption as applied to the effects of Bordeaux mixture.

Potometer experiments.—In attempting to secure leaves satisfactory for the work, some incidental observations were made which are of interest. The work was begun during the winter, so that greenhouse-grown plants alone were available. Furthermore, in this work with potometers, Bordeaux mixture alone has been used by us. Testing leaves of squash (*Cucurbita sp.*), *Pelargonium zonata*, and *Phytolacca*, also shoots of potato and *Irescene*, as to their behavior under the conditions required, it was found that of comparable leaves, sprayed and unsprayed, invariably the sprayed leaves were the first to wilt. This might be attributed either to an injurious action of the spray or to a greater water consumption. That the last mentioned is the more probable explanation finds confirmation through a special observation on the potato. Owing apparently to some stoppage of the vascular system, abscised potato shoots are unsuitable for potometer work, wilting in a comparatively short time even when cut under water; and sprayed potato shoots wilt more quickly than unsprayed, thus pointing

to a more rapid water elimination after spraying. Potted potato plants from which the shoots were cut withstood the fungicide satisfactorily.

Leaves of the large elephant's ear (*Caladium sp.*) proved unsatisfactory on account of the excessive "bloom," which interfered with the proper application of the spray. Canna leaves were similarly unfavorable, and leaves of the calla lily wilt soon after abscision.

It has been stated above that the leaves of the castor bean proved most satisfactory in the potometer work. The experiments with these leaves were carried out in the open, except as otherwise noted, during the early fall. The plants were arranged for standardization and for subsequent observation at distances of about ten feet apart on an exposed lawn uniformly sodded. No readings were made until the leaves had become adjusted to the conditions. Observations were made at frequent intervals when the water loss was rapid, in order to maintain the water column at a fairly uniform level, so that many of the data given in the tables which follow represent summations of several successive readings. Three series of potometer experiments were made, each series embracing six leaves, but in one series, accidents to some of the leaves, and the necessity of substituting new ones after the experiment began, resulted in such a shortening of the standardization intervals that it was thought necessary to discard the results, although they were in the same direction as the others obtained.

The data are presented in full in the tables and all available data are used in computing the relations given. The relations may be more conveniently expressed if we first divide the leaves into classes, designated by letters, as follows:

$A-$, three leaves (i. e., the transpiration quantities of these) in the standardization interval before spraying.

$A+$, the same three leaves as in $A-$, but for any interval after spraying.

B, three control leaves (unsprayed throughout) during the standardization interval.

B', the control leaves as in B, after standardization.

The ratio $\frac{A-}{B}=Q$ is to be compared with the ratio $\frac{A+}{B'}=Q'$.

If Q' is greater than Q, then the spraying facilitates transpiration; if less, then the converse is true. If no accidents occurred during the experiments, $\frac{A-}{B}$ would, of course, be a constant quantity, each term referring properly to the summed transpiration quantities for three leaves during the standardization interval. Accidents are unavoidable, however, during the subsequent observations, and whenever these occur it is necessary to compute a new value of Q' for any particular "run" in which the accident occurs. The only consideration then is to have the same leaves (i. e., their summed transpiration quantities) in the ratios before and after standardization. If, for example, it is necessary to use a ratio, Q', of Nos. 1 and 3 to Nos. 2, 4, and 6 after spraying, then the new value of Q (in the standardization interval) for comparison must also be computed with Nos. 1 and 3 against Nos. 2, 4, and 6.

TABLE I

EFFECT OF FILMS OF BORDEAUX MIXTURE ON TRANSPIRATION OF STANDARDIZED CASTOR BEAN LEAVES; DATA FOR DAY PERIODS

No. of leaf	1	2	3	4	5	6	Ratio
Transp. 12:30–2:30 P. M., 1st day before spraying	$A-$ 10.8	B 17.3	$A-$ 28.5	B 45.9	B 33.8	$A-$ 33.6	$\frac{A-}{B}=\frac{72.9}{97.0}$
Transp. 3:12–5:00 P. M., 1st day after spraying	$A+$ 7.6	B' 20.4	$A+$ 23.2	B' 26.2	B' 26.1	$A+$ 39.5	$\frac{A+}{B'}=\frac{70.3}{72.7}$
Relation, sprayed to unsprayed, 1st day	Rate changed from $\frac{72.9}{97.0}$ (=.75) to $\frac{70.3}{72.7}$ (=.97)						
Transp. 8:12–9:48 A.M., 2nd day after spraying	$A+$ 10.2	B' 37.7	$A+$ 63.1	B' 32.3	B' 29.7	$A+$ 67.1	$\frac{A+}{B'}=\frac{140.4}{99.7}$
Relation, sprayed to unsprayed, 2nd day, *a*.	Rate changed from $\frac{72.9}{97.0}$ (=.75) to $\frac{140.4}{99.7}$ (=1.41)						
Transp. 11:16–11:53 A. M., 2nd day after spraying	$A+$ 4.9	B' 5.9	$A+$ 31.5	B' 13.8	B' 7.5	$A+$ 25.3	$\frac{A+}{B'}=\frac{61.7}{27.2}$
Relation, sprayed to unsprayed, 2nd day, *b*.	Rate changed from $\frac{72.9}{97.0}$ (=.75) to $\frac{61.7}{27.2}$ (=2.3)						

TABLE II

EFFECT OF BORDEAUX MIXTURE ON TRANSPIRATION OF STANDARDIZED CASTOR BEAN LEAVES; DATA FOR DAY AND NIGHT PERIODS

No. of leaf.	1	2	3	4	5	6	Ratio
Transp. 4:04–5:25 P. M., 1st day before spr.	$\frac{A-}{7.5}$	$\frac{B}{7.6}$	$\frac{A-}{10.9}$	$\frac{B}{11.7}$	$\frac{A-}{7.6}$	$\frac{B}{9.4}$	
Transp. 8:21–11:17 A. M., 2nd day before spr.	$\frac{A-}{20.2}$	$\frac{B}{30.7}$	$\frac{A-}{32.4}$	$\frac{B}{40.9}$	$\frac{A-}{23.3}$	$\frac{B}{17.9}$	
Total transp. before spr.	$\frac{A-}{27.7}$	$\frac{B}{38.3}$	$\frac{A-}{43.3}$	$\frac{B}{52.6}$	$\frac{A-}{29.9}$	$\frac{B}{27.3}$	$\frac{A-}{B}=\frac{101.9}{118.2}$
Transp. 12:30–4:50 P. M., 1st day after spr.	$\frac{A+}{36.7}$	$\frac{B'}{42.6}$	$\frac{A+}{62.9}$	$\frac{B'}{50.2}$	$\frac{A+}{41.0}$	$\frac{B'}{30.2}$	$\frac{A+}{B'}=\frac{140.6}{123}$
Relation, sprayed to unsprayed, 1st day	Rates changed from $\frac{101.9}{125.2}$ (=.86) to $\frac{140.6}{123}$ (=1.14)						
Transp. 8:56 A. M., to 4:44 P. M., 2nd day aft. spr.	$\frac{A+}{20.7}$	$\frac{B'}{—}$	$\frac{A+}{28.9}$	$\frac{B'}{18.2}$	$\frac{A+}{17.0}$	$\frac{B'}{12.1}$	$\frac{A+}{B'}=\frac{66.6}{30.3}$
Relation, sprayed to unsprayed, 2nd day	Rates changed from $\frac{101.9}{79.9}$ (=1.28) to $\frac{66.6}{30.3}$ (=2.2)						
Transp. 10:27 A. M., to 3:40 P. M., 3rd day aft. spr.	$\frac{A+}{8.4}$	$\frac{B'}{—}$	$\frac{A+}{11.2}$	$\frac{B'}{4.4}$	$\frac{A+}{7.8}$	$\frac{B'}{3.5}$	$\frac{A+}{B'}=\frac{27.4}{7.9}$
Relation, sprayed to unsprayed, 3rd day	Rates changed from $\frac{101.9}{79.9}$ (=1.28) to $\frac{27.4}{7.9}$ (=3.46)						
Transp. 9:58 A. M., to 4:42 P.M., 4th day aft. spr.	$\frac{A+}{—}$	$\frac{B'}{—}$	$\frac{A+}{22.1}$	$\frac{B'}{14.1}$	$\frac{A+}{15.3}$	$\frac{B'}{7.9}$	$\frac{A+}{B'}=\frac{37.4}{22.0}$
* Relation, sprayed to unsprayed, 4th day	Rates changed from $\frac{74.2}{79.9}$ (=.93) to $\frac{37.4}{22.0}$ (=1.7)						
Total transp. after spraying	$\frac{—}{—}$	$\frac{—}{—}$	$\frac{A+}{125.1}$	$\frac{B'}{86.9}$	$\frac{A+}{81.1}$	$\frac{B'}{53.7}$	$\frac{A+}{B'}=\frac{206.2}{140.6}$
Relation, sprayed to unsprayed, totals	Rates of totals changed from $\frac{74.2}{79.9}$ (=.93) to $\frac{206.2}{140.6}$ (=1.47)						
Transp. 5:30 P. M., to 8:21 A. M., 1st night bef. spr.	$\frac{A-}{6.9}$	$\frac{B}{6.3}$	$\frac{A-}{9.0}$	$\frac{B}{11.2}$	$\frac{A-}{6.4}$	$\frac{B}{—}$	$\frac{A-}{B}=\frac{22.3}{17.5}$
Transp. 4:50 P. M., to 8:40 A. M., 1st night aft. spr.	$\frac{A+}{6.9}$	$\frac{B'}{7.8}$	$\frac{A+}{2.7}$	$\frac{B'}{7.5}$	$\frac{A+}{6.2}$	$\frac{B'}{3.5}$	$\frac{A+}{B'}=\frac{15.8}{15.3}$
Relation, sprayed to unsprayed (night)	Rate changed from $\frac{22.3}{17.5}$ (=1.27) to $\frac{15.8}{15.3}$ (=1.03)						
Transp. 3:45 P. M., to 9:30 A. M., 2nd night aft. spr.	$\frac{A+}{20.0}$	$\frac{B'}{—}$	$\frac{A+}{15.1}$	$\frac{B'}{4.9}$	$\frac{A+}{5.8}$	$\frac{B'}{4.2}$	$\frac{A+}{B'}=\frac{40.9}{4.9}$
Relation, sprayed to unsprayed (night)	Rate changed from $\frac{22.3}{11.2}$ (=2.0) to $\frac{40.9}{4.9}$ (=8.34)						

* For this "run" the plants were transferred to a room in the building.

Summarizing the data for the rates in table II, day intervals, we find that $Q:Q'$, in the successive periods, as .86:1.14, as 1.28:2.2, as 1.28:3.46, and as .93:1.7. If we make the ratio before spraying equal in each case, to 1.0, then the value for the periods after spraying in the successive day intervals are respectively 1.33, 1.72, and 1.83. These differences in rate are so marked and consistent as to outweigh all considerations of individual differences, as disclosed by a detailed study of the figures in table II. It will also be noted that the less extensive data from table I are confirmatory; thus $Q:Q'$, in the successive intervals, as .75:.97, as .75:1.41, and as .75:2.3. On the basis of 1.0 for the ratio before spraying, we have for the periods after spraying, respectively, 1.29, 1.88, and 3.07. From the records of the potometer experiments it is obvious that only one conclusion may be drawn, namely, that the rate of transpiration is materially increased after spraying.

Some points relative to environmental conditions, however, require special mention, and certain suggestive results must be left for further experimental study. Attention has been drawn to the fact that, in general, the potometer experiments were conducted in the open, during early October. During the last days of the work, cooler weather and danger from rain made it desirable to transfer the potometers to a room in the building, and the data for the third and fourth days after spraying, table II, were secured under these new conditions. In this room the shades were drawn and every precaution taken to secure uniformity. It will be noted that while the order of results is in the same direction as for the lawn exposure, the ratio is even higher than the average. No "shading action" of the Bordeaux, as postulated by Schander (18), could be considered a factor of importance in this case.

The results in the laboratory suggest, further, that the ratio of sprayed to unsprayed will vary considerably with the conditions. Before removing the potometers to the laboratory, the night temperatures were so low that two night "runs" (including the interval from about 6 P. M. to 8 A. M.) were necessarily excluded on account of leakage. Other night "runs," as shown in the tables, indicate the probability that under certain conditions unfavorable for evaporation, the surface

film may actually effect a diminution in the rate of transpiration, although the transpiration data do not suffice to warrant more, at present, than an impression. In fact, the night "runs" should be considered apart from those of the day, for the latter are much more satisfactory.

Experiments with potted plants.—The experiments with potted tomato plants were divided into two series which were consecutive in time, and different only with respect to the substances applied to the leaves. As far as has been ascertained, this is the first time that tomato plants have been used in such work, but in our experience they are more satisfactory than potatoes. In the first series (table III) 30 plants were used, in lots of 10 each, for the applications of (1) strong Bordeaux mixture, (2) weak Bordeaux, and (3) controls. In the second series (table IV, V) 80 plants were used in 8 lots, and the substances employed as sprays or dusts are noted in the tables. In the second series it is to be noted that there are 3 substances of the nature of films ($Ca(OH)_2$, $Al(OH)_3$, and lime-sulphur), 1 true suspension (clay), and 3 powders (charcoal, $CaCO_3$, and powdered $Al(OH)_3$).

The methods of procedure involved in these experiments have already been outlined. It is necessary to add, however, that the plants used were about 12 inches high and as uniform in size as could be obtained. It was not possible satisfactorily to standardize plants for an experiment extending over several weeks: and it was necessary to rely in part upon numbers, and in part upon a rigidly accurate method of selecting the individual in each lot to eliminate any errors. The method of selection consisted in getting together 8 plants so similar in size and vigor that no choice could be made between them, then distributing these at random to the 8 lots, this being continued until each lot embraced 10 plants.

In each case the experiments extend over 2 periods. At the close of the first period the plants were shifted in position and a second application of the spray mixture or dust was given. With the conclusion of the experiment the green weights of all plants were taken, thus enabling us to determine, in addition to the total transpiration quantities, the amount of transpiration per gram of green substance.

TABLE III

THE EFFECTS OF BORDEAUX MIXTURE ON THE RATE OF TRANSPIRATION; DATA IN GRAMS FOR 30 POTTED TOMATO PLANTS

	1st period Oct. 18 to Nov. 4		
Covering	Strong Bord.	Weak Bord.	Check
Plants nos.	1–10	11–20	21–30
Transpiration quantities	702 684 665 630 625 710 640 445 645 560	681 651 540 585 857 440 585 648 645	390 555 375 525 395 465 415 365 490 545
Total	6306	5622	4520
Ave. per plant	630.6	625	452.0
	Second period Nov. 5–15		
Covering	Strong Bord.	Weak Bord.	Check
Plants nos.	1–10	11–20	21–30
Transpiration quantities	571 559 575 574 515 740 514 570 495 546	628 549 442 603 720 453 499 534 702	356 554 368 518 385 420 437 417 439 564
Total	5659	5130	4458
Ave. per plant	565.9	570	445.8

TABLE III (*Continued*)

THE EFFECTS OF BORDEAUX MIXTURE ON THE RATE OF TRANSPIRATION; DATA IN GRAMS FOR 30 POTTED TOMATO PLANTS

Green wts. of plants used.

Plants nos.	1–10	11–20	21–30
Green weights in grams	55	80	49
	58	58	63
	51	41	46
	39	51	56
	50	60	39
	67	45	53
	46	40	48
	46	53	40
	39	74	48
	49	..	55
Total	500	502	497
Ave. per plant	50.0	55.8	49.7

TABLE IV

THE EFFECT OF VARIOUS SPRAYS AND DUSTS ON THE RATE OF TRANSPIRATION; DATA IN GRAMS FOR 80 POTTED TOMATO PLANTS. 1ST PERIOD, OCT. 25 TO NOV. 8

Covering	$Ca(OH)_2$	$Al(OH)_3$	Clay	$Al(OH)_3$ pwd.	Charcoal	$CaCO_3$	Lime-sulfur 1–25	Check
Plants nos.	30–39	40–49	50–59	60–69	70–79	80–89	100–109	90–99
Transpiration quantities	431	394	345	416	378	436	508	352
	437	370	333	370	460	353	414	323
	435	386	430	393	374	315	474	461
	411	383	383	383	460	510	354	443
	358	329	347	645	273	375	526	490
	372	377	520	449	467	346	421	309
	314	398	437	365	320	471	352	330
	416	410	560	531	359	361	346	323
	375	517	362	408	386	456	285	343
	485	460	452	412	331	364	402	317
Totals	4034	4024	4169	4372	3808	3987	4082	3691
Ave. per plant	403.4	402.4	416.9	437.2	380.8	398.7	408.2	369.1

TABLE V

THE EFFECTS OF VARIOUS SPRAYS AND DUSTS ON THE RATE OF TRANSPIRATION; DATA IN GRAMS FOR 80 POTTED TOMATO PLANTS. 2ND PERIOD, OCT. 25 TO NOV. 8.

Covering	$Ca(OH)_2$	$Al(OH)_3$	Clay	$Al(OH)_3$ pwd.	Charcoal	$CaCO_3$	Lime-sulfur 1–25	Check
Plants nos.	30–39	40–49	50–59	60–69	70–79	80–89	100–109	90–99
Transpiration quantities	494	812	624	665	573	560	846	590
	463	587	582	601	610	543	641	569
	592	653	693	609	530	553	667	564
	539	587	615	654	744	700	622	701
	604	654	594	754	556	512	680	764
	457	614	579	606	617	552	616	546
	544	665	694	476	569	706	604	496
	647	605	753	704	664	585	478	558
	597	806	579	641	596	620	601	514
	582	810	590	711	562	617	428	601
Totals	5509	6793	6303	6421	6021	5948	6183	5903
Ave.	550.9	679.3	630.3	642.1	602.1	594.8	618.3	590.3
*Transp. per gm.	11.8	12.2	12.3	11.4	13.1	12.0	12.8	12.1

Green wts. of plants 30–109 at close of 2nd period.

Green weights in grams	39	71	46	58	49	50	56	58
	39	43	47	52	43	47	51	47
	49	58	51	45	48	43	42	38
	42	53	49	79	35	53	53	57
	60	56	54	77	35	53	67	53
	43	56	58	47	59	51	40	56
	41	53	52	47	46	57	52	35
	50	55	50	54	48	47	36	46
	60	58	48	47	52	43	49	46
	43	54	59	55	43	51	39	51
Totals	466	557	514	561	458	495	485	487
Ave. per plant	46.6	55.7	51.4	56.1	45.8	49.5	48.5	48.7

* Computed on the basis of green weights at the close of second period.

An examination of the data in the several tables involved in the pot experiments serve to indicate that while there is a certain amount of individual variation in the transpiration quantities of the various plants in any group, general conclusions seem to be warranted. The individual variations in transpiration in the Bordeaux series are in closer accord with the variations in green weight of the plants used than are those in the other series. Taking all factors into consideration, a film of Bordeaux mixture is found to facilitate transpiration. Other films and dusts employed do not seem to affect the rate of transpiration to the same extent.

In a consideration of the results in detail it is to be noted that the Bordeaux series (table III) is not strictly comparable with the other (tables IV, V), since they were not conducted simultaneously. If the transpiration in grams per gram of green weight for the control (Bordeaux series) is represented by 100, then the rate for weak Bordeaux on this basis is 113.2, and the rate for strong Bordeaux is 125.43. The differences are in the same direction, but not so great as those obtained with the potometer experiments. The use of both weak and strong Bordeaux mixture materially strengthens the conclusions to be deduced.

The series which gives the results with other sprays and dusts is not so easily interpreted. The transpiration quantities vary slightly on either side of the control, and no covering gives a negative difference (contrasted with the control) greater than six per cent (this is the case of $Al(OH)_3$), or a positive difference greater than about eight per cent (charcoal).

These slight average differences may be no more than would be explained by the possible experimental error; but it is of interest to perceive that, with the exception of clay, those surface applications which give lower values than the control are those which might diminish the absorption of heat in direct sunshine. The results might then be the resultant of two factors, (1) the direct effect of the surface film or dust on the rate of water loss, and (2) the indirect effect exerted through a modification of the temperature of the leaf.

Accepting as a general conclusion an acceleration of transpiration (specifically in the castor bean and in the tomato)

as a result of an application of a film of Bordeaux mixture, the following questions arise: (1) What is the physical or chemical basis of the increased evaporation from plant surfaces covered with Bordeaux mixture? (2) Is the increased evaporation in any way related to the increased vitality or longevity of sprayed leaves? Neither of these questions may be answered intelligently at present. With respect to the first, we have arranged experiments to determine the effects of Bordeaux on the passage of water vapor through non-living membranes; but the results are thus far conflicting, due possibly to the fact that we have not yet used membranes which are satisfactory analogues of leaves. Experiments in this direction will be reported later. No relation of transpiration to increased longevity can be foretold, although it seems possible that the highest efficiency equilibrium relation of longevity may involve, in certain plants, a relatively high transpiration rate as either a direct or an indirect factor. No answer to the question will be satisfactory until a further study of other effects ("stimulation," increased "assimilatory activity," etc.) of Bordeaux mixture shall have been made.

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Explanation of Plate

PLATE I

Potometer used in the transpiration experiments showing burette connected with the side arm flask, and abscised leaf of *Ricinus* cemented into the mouth with plastolina.

DUGGAR AND COOLEY—TRANSPIRATION

SOME PURE CULTURE METHODS IN THE ALGÆ

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Introduction

Too much confidence has frequently been placed by algologists in their ability to recognize a given species of alga among varying numbers of other species, and in the various forms which it may assume—a fact which has led to much confusion and error, especially among members of the *Protococcales*. While it is now definitely known that in a number of algæ a single species may present markedly dissimilar appearances, either as a result of varying environmental conditions, or because of the presence in the life history of several unlike stages, it is certain that much of the so-called *polymorphism*, or *pleomorphism*, of algæ finds its explanation in inadequate methods of study. It is becoming recognized that for life history studies in the algæ it is necessary to employ cultures free from other species of algæ. Even in cases where this is not, on first thought, necessary, as in the large, filamentous forms, it should be observed, for the possibility of introducing spores or sporelings of closely allied species is by no means excluded in all cases. Gratifying progress has already been made by some algologists, working especially with members of the *Volvocales* and *Protococcales*, and it seems reasonably certain that the originally chaotic condition existing in the latter will be ultimately reduced to complete order by a careful observance of the necessity of working with pure cultures, or at least cultures containing but a single species of alga. In life-history studies where physiological differences between species are to be investigated, it is especially desirable and indeed necessary to employ pure cultures.

Certain species of algæ, especially representatives of the *Chlorophyceæ*, have been much used in physiological investigations—chiefly those concerning themselves with various

phases of nutrition. With the development of a clearer understanding of the activities and life processes of the various micro-organisms, the necessity of working with rigorously pure cultures has become more and more evident. It is now generally appreciated that, in most cases, valid conclusions as to the physiology of a particular organism cannot be drawn with certainty where one or more foreign organisms have been present in the cultures. There can be no doubt that the frequent contamination of cultures of algæ with bacteria, and even with fungi, has, in many cases, detracted markedly from the value of painstaking and otherwise careful physiological investigations. The readiness, however, with which many algæ lend themselves to experimental purposes—on account of their small size and ease of handling and culture—will always make them favored objects of study; and it appears desirable at this time to bring together some of the experiences of the author in the preparation of pure cultures of algæ, with the hope that suggestions may be gained from them by those who desire to obtain such cultures for one purpose or another.

An unfortunate use of the term "pure culture" has come into more or less general use and has frequently led to confusion and ambiguity. As used by many authors, it means simply a culture of a single species of alga not necessarily free from bacteria and fungi. Where the presence of other organisms is not specifically mentioned, it is clear that the above usage of the term may lead to serious misunderstandings. Indeed, it remains for the reader, in many instances, to decide for himself—from the technique employed—whether a culture of an alga free from all other organisms or only from other species of algæ is meant. It is to be hoped, therefore, that the term pure culture shall come to have the same clearly defined meaning when used in connection with the algæ that it has long had in the fungi and bacteria. In the following report the term is used to signify a culture of a single species of alga free from all other organisms.

HISTORICAL

Although incidental references to pure culture technique in the algæ are frequently found in the literature, relatively few contributions have appeared which deal extensively with the

subject, or which outline in detail the methods employed. Beyerinck, in 1890 (4, 6), appears to have been the first to succeed in isolating species of algæ in pure culture. Ditch water, boiled with ten per cent gelatin, and cooled, was mixed with a drop of water rich in protococcoid algæ, poured into dishes, and allowed to cool. Numerous minute algal colonies appeared in course of time, and the number of bacterial colonies developing was so small that successful transfers of *Scenedesmus acutus* Meyen and *Chlorella vulgaris* Bey., were made, both organisms being subsequently cultured on a variety of media. In addition, the gonidia of *Physcia parietina* were obtained pure. Small pieces of the lichen thallus, carefully washed, were placed on solid gelatin plates. Those which showed themselves to be free from foreign organisms were transferred to gelatin plates containing malt-extract, the fragment being first torn to bits with needles and then dragged over the sterile surface. In a few days, small colonies of the algal symbiont appeared from which successful transfers were made. In a later paper (5), Beyerinck adds *Stichococcus major* and a second species of *Chlorella* to the list of algæ previously cultured in a state of purity, the technique, in general, being the same.

Miquel (16) was the first to isolate a diatom in pure culture. Subsequently, Richter (20, 21) isolated *Nitzschia Palea* (Kütz.) W. Sm., and *Navicula minuscula* Grun., by the use of synthetic agar plates. Attention is called by this author to the importance of using agar which has previously been washed to free it from soluble impurities. A mixture of diatoms and other algæ was placed on the surface of washed agar plates, and from the impure diatom colonies which developed transfers were made to other plates until at length pure cultures were obtained.

In his isolations of certain protozoa in pure culture, Ogata (18) also obtained *Polytoma uvella*. While his method seems unnecessarily complex, it is of interest here. Sterile capillary tubes were filled in part with a column of sterile water, and subsequently a column containing the organisms was added below, care being taken not to separate the two by air. Both ends of the tube were then sealed. After sufficient time had elapsed for the movement of the motile *Polytoma* cells from the lower column into the upper sterile one, the tube was broken in

the region of the upper column. The lower portion was discarded, and the upper one was sealed, subsequently transferred to a sterile medium, and broken to permit the organisms, free from contaminations, to enter the medium and begin their development.

By the gelatin plate method, Krüger (13) prepared pure cultures of two new organisms—*Chlorella protothecoides* and *Chlorothecium saccharophilum*—obtained from the exudation of *Populus alba*. Tischutkin (23) lists representatives from about eighteen genera of algæ—including diatoms, green, and blue-green forms—as having been obtained in pure culture by the agar plate method. After three or four successive dilutions in liquid one per cent agar, the organisms were plated in Petri dishes. The filamentous forms he washed in sterile water, cut into short segments, and transferred to the liquid medium. The methods given by Ward (24) include plating in agar and silicic acid jelly, though as a whole the methods are applicable for the separation of algal species rather than for their isolation in pure culture. This is especially true of the plaster of Paris, and precipitated calcium carbonate methods. Gonidia from *Xanthoria parietina*, and *Gasparinia murorum* (Hoffm.) Tornab., together with *Pleurococcus vulgaris* and *Scenedesmus caudatus* were obtained in pure culture by Artari (1). Chodat and Goldflus (8), by the use of pieces of sterilized unglazed porcelain in contact with a mineral nutrient solution, claim to have isolated a species of *Nostoc* in pure culture. The procedure was a simple one, consisting in repeated transfers to fresh sterile plates until a pure culture was at length obtained.

Several years later Chodat and Grintzesco (9) reported that by essentially the same method, *Oocystis elliptica*, *Dictyosphærium pulchellum*, *Kirchneriella lunaris*, *Rhaphidium polymorphum*, *Pediastrum tetras*, *Scenedesmus acutus*, *Pleurococcus vulgaris*, *Hæmatococcus lacustris*, and *Chlorella vulgaris* had been obtained in pure culture. In cases where the number of algal individuals is small, but the bacteria and fungi relatively abundant, the authors point out the desirability of first increasing the number of the former by introducing the mixture into a mineral nutrient solution favorable for the growth of the algæ but not so for the fungi. Where filamentous forms are

concerned, the authors state that it is necessary to begin with the zoöspore, as a pure culture from filaments is extremely difficult to obtain. My own experience does not bear out this statement in all cases as it was found that especially among the *Ulotrichales* pure colonies were regularly and easily obtained from filaments.

Artari in 1902 (2) reports the isolation of *Chlorococcum infusionum* and *Scenedesmus caudatus* in pure culture. Chick (7) attempted to isolate *Chlorella pyrenoidosa* through the use of sterilizing agents such as hydrogen peroxide and sunlight. These trials, however, did not prove successful, as the alga failed to show a resistance sufficiently greater than that of the bacteria to make possible a successful separation. The isolation was finally attained by placing a few drops of water containing the organism on a sterile synthetic agar plate, and spreading the same over the surface with a brush. The same brush was used to distribute sterile water drops over the surface of other plates, no additional algal material being added. From the later dilutions pure colonies were obtained. Frank (10) was unable to obtain pure cultures of *Chlamydomonas tingens* by the agar plate method.

Jacobsen (11) reports the isolation of *Chlorogonium* and *Polytoma* in pure culture. This author made use of an interesting method of separation of algal species based on their different degrees of resistance to drying. Discs of filter paper, on which drops of water containing *Spondylomorum* and *Chlamydomonas variabilis* had been placed, were dried in an incubator at 28°C. After twenty-four hours, the discs were placed in a suitable medium, but only the *Chlamydomonas* species developed, *Spondylomorum* having been killed. *Chlorogonium euchlorum* and *Polytoma uvella* also showed themselves very sensitive to drying, whereas *Chlamydomonas* usually survived the desiccation. Old cultures of *Chlorogonium euchlorum* proved to be very resistant owing to the presence of zygospores which had been formed by the conjugation of gametes.

While reference might be made to a number of other investigations which deal in an incidental way with pure culture technique, it is believed that those given will serve to indicate, in a general way, the present status of the subject. (For further

information the reader is referred to Moore (17), Richter (21), Küster (14), and others.) It is apparent that the large majority of forms isolated in pure culture belong to the *Protococcales*. Only a few of the filamentous forms, several diatoms, and but one or two species of the blue-green algæ have thus far yielded to pure culture technique.

PURE CULTURE TECHNIQUE

GENERAL

Algæ, generally speaking, are provided with a more or less highly developed exterior mucilaginous investment which may be either a distinct, separable sheath, as in many of the *Cyanophyceæ*, or merely a gelatinization resulting either from a modification of the external portion of the membrane, or from an internal secretion, as in some of the desmids. In general, also, algæ are slow growing as compared with many fungi. In these two characteristics most of the difficulties encountered in pure culture technique among the algæ find their explanation.

Among the fungi, spores with non-gelatinous walls are readily obtainable in a majority of the forms, and usually in great abundance. When such spores are plated in the way ordinarily employed in bacteriological technique, a large number of colonies free from bacteria are usually obtained. Among the algæ, however, such non-gelatinous, resistant spores are, if produced at all, generally present only in small quantities. When vegetative algal cells are plated on a suitable medium, algal colonies will often be obtained, but they usually form the nucleus of a larger bacterial colony which has developed from the bacteria adhering to the gelatinous surface of the algal cell. Among those fungi in which spores are not readily obtained, an isolation in pure culture may frequently be effected by allowing the fungus to grow on a suitable medium until the hyphæ have outstripped the bacteria in their growth, at which time pure mycelial transfers may be made from the terminal portions. If, however, a like procedure is attempted with the algæ it will usually be found that the bacteria adhere tenaciously to the surface of the growing filaments and are carried

along by the lengthening filaments. Except in rare cases, nothing is to be gained by this procedure in the algæ. The task of isolating pure cultures of algæ, therefore, becomes an individual problem for almost every species as it necessitates at once the determination of the period in the life history of any form at which the cells are free from bacteria or at which time the bacteria can be removed by one means or another. Having found a stage in which the alga is bacteria-free, it is of importance next to be able to bring about this stage more or less at will in order that the alga may be utilized when available. To obtain the above preliminary information, nothing is more serviceable than the usual plating method on a suitable medium.

The Medium.—The requirements of a suitable solid medium for algal isolating purposes are, that it remain liquid down to a temperature at which delicate algal cells are not injured; that it be suitable for the growth of algæ, and as unfavorable as possible for the growth of bacteria and fungi. For this purpose nothing was found so serviceable as the following, the mineral ingredients being in the proportions recommended by Moore (17):

Agar	10.0 grams
NH_4NO_3	0.5 gram
$MgSO_4 . 7H_2O$	0.2 gram
K_2HPO_4	0.2 gram
$CaCl_2$	0.1 gram
$FeSO_4$	trace
Dist. H_2O	1000 cc.

The agar should be carefully washed, first in a stream of tap water and then in distilled water, as pointed out by Richter (20). An agar so prepared will remain liquid down to about 34.5–35°C., and experience has shown that even the most delicate algal cells are uninjured by the short exposure to this temperature necessary in the plating process. From six to eight cc. of agar in a Petri dish eight cm. in diameter is a suitable quantity with which to plate. Larger quantities so thicken the layer of agar in the dish that the higher powers of the microscope, with their objectives of short focal length, cannot be used in locating small developing colonies.

Material to be Plated.—The alga to be plated should be collected with as little adhering foreign matter as possible. If it is a filamentous form which can be manipulated with a platinum needle, it can be materially cleansed by washing in sterilized nutrient solution such as is used in the preparation of the agar. If the alga is a unicellular form, little can be done in the way of preliminary cleansing. Dilutions are made in the usual manner, the degree depending upon the number of algal organisms present. The degree of dilution will depend in part, also, upon the number of bacteria and fungi present as determined by microscopic examination. It must be remembered that the algæ grow more slowly than most bacteria and fungi, and that unless the dilution, from the standpoint of the total number of organisms present, is great enough, the spread of bacterial and fungal colonies may be so great as to make the transfer of the later-appearing algal colonies impossible without contamination.

The material should be introduced into the tube of liquid agar while the latter is still a few degrees above its congealing point, in order that the inoculated tube may be vigorously shaken for some time before its contents are poured into the Petri dish. In this way the algal cells are freed of large numbers of either accidentally or regularly adhering bacteria.

Incubation and Transference.—The plates, after the agar has solidified, should be turned upside down in order to prevent the moisture which condenses on the cover from dropping, and spreading bacteria over the surface of the agar. Failure to do this often renders large numbers of platings worthless. The most favorable place to keep plates is in the light of a north window; and, as plates frequently remain under observation for many weeks, it is further desirable to have them in a glass case to prevent outside contamination. In general it is not advisable to cover the plates with bell jars, as it increases the humidity in the Petri dishes and accelerates the growth of moulds present as contaminations. The plates should be examined frequently and when rapidly spreading colonies of fungi or bacteria appear, these should be dissected out in order to save the remainder of the plate.

The length of time necessary for the appearance of the algal

colonies varies greatly with the species, from one to three or four weeks usually being required, depending upon the particular form. In most cases it is not possible to wait until the algal colonies can be seen macroscopically because spreading bacterial and fungal colonies usually encroach on the former to such an extent that a pure transfer is no longer possible. It becomes necessary, therefore, to look the plates over from time to time with the compound microscope in order to locate algal colonies in very early stages of development. For this purpose a 12 mm. objective is extremely serviceable, as its focal length is of sufficient magnitude to enable one to use it through the agar layer and glass bottom of a Petri dish and at the same time obtain a magnification considerably greater than that afforded by the ordinary low-power objective. The colonies located are conveniently marked by placing a small ink dot directly opposite them on the bottom of the Petri dish. Transfers should be made to agar slants by means of a minute platinum-foil spatula with which the agar directly over the ink dot can be neatly dissected out and transferred to the slant. It is not possible, in most cases, to make successful transfers with a platinum needle because the algal colony is usually composed of firmly cohering cells and, even in repeated attempts, not a single individual will adhere to the needle. Since many of the colonies are in the deeper strata, it is well to spread out the transferred agar fragment in a thin sheet in order to expose the contained algal cells directly to the air. Unless this is done, subsequent development in the slant may be extremely slow. Although bacteria grow slowly on this synthetic agar, their development is usually sufficient in a week to indicate whether the transfer has been successful or not. The purity of the culture may be further tested by making transfers to media more suitable for bacterial growth.

With this brief preliminary consideration of some of the more general phases of pure culture technique in the algæ, the isolation of single species will now be considered and attention called to the special problems and the technique involved in their isolation.

SPECIFIC

CHLOROPHYCEÆ

Chlamydomonas pisiformis Dill forma *minor* Spargo.—*Chlamydomonas* species frequently occur in water rich in organic materials, and teeming with bacteria. When the alga was in the resting condition, the mucilaginous cell walls were found so impregnated with bacteria as to render isolation in pure culture impossible. Platings with motile cells, however, showed that the latter were absolutely free from regularly adhering bacteria, but the number of bacteria present rendered the plates worthless. Then the gelatinous masses of resting cells were repeatedly washed with sterile water and finally placed in distilled water where, after twelve to twenty-four hours, zoöspores appeared in great abundance and congregated on the side of the vessel nearest to the light. A minute portion of this liquid containing the zoöspores was removed with a fine capillary tube and introduced into a tube of liquid agar and plated. In platings thus made, numerous colonies of *Chlamydomonas* appeared and the number of bacterial colonies was so small that a large number of successful pure transfers were made.

Where the number of available motile cells is small and it is important that isolations be made from these, a modification of the method used by Barber (3) in the isolation of yeasts and bacteria was frequently used to advantage. A large number of small, capillary pipettes were made and sterilized. After locating the cell or cells desired, they were removed with a pipette while being observed under the microscope, and transferred to a drop of sterile nutrient solution or water. This process was repeated until it was certain that the number of bacteria had been reduced sufficiently to admit of successful plating. They were then taken up again by means of a sterile pipette, transferred to a tube of liquid agar, and plated. Numerous pure cultures were obtained in this way.

Stichococcus bacillaris Näg., and *S. subtilis* (Kütz.) Klercker.—Preliminary platings with these forms showed that the cells, as obtained from the soil, yielded abundant bacteria-free colonies, and the problem of isolation became one of merely obtaining clean material and diluting sufficiently. Both of these species

of *Stichococcus* are soil-inhabiting and can be obtained—practically free from other algæ—on flower pots and greenhouse soils. The former species, because of its minute cells and the readiness with which the filaments resolve themselves completely into their constituent cells when placed in water, is a particularly easy one to obtain in pure culture. Rich material may be diluted until plates obtained from it show a sufficiently small number of bacterial colonies to admit of pure transfers and yet enough algal colonies for a number of transfers. *S. subtilis* is a larger species and the cells remain attached in rather long filaments. However, with vigorous shaking and previous teasing apart with needles, a sufficient number of single cells and small fragments of filaments are introduced to make possible numerous successful isolations. The washing of the cells to remove adhering bacteria can, in these species and many others, be largely accomplished by introducing the raw material into test-tubes containing sterile mineral nutrient solution or water, stoppering, and shaking vigorously. Direct transfers from these to liquid agar, or to tubes of sterile water for further dilution, may then be made. This procedure frequently enables one to make successful platings where the direct transfer of raw material to liquid agar results in constant failure.

Chlorella vulgaris Bey., and *Chlorella sp.*—Both of these species were isolated from soil in the open. An exterior gelatinous investment is, as in the two above mentioned species of *Stichococcus*, conspicuously absent, and preliminary experiments demonstrated that a large number of the vegetative cells were freed from all accidentally adhering bacteria by being shaken in the liquid agar before plating. The problem of isolating these species again becomes one of clean material and sufficient dilution. Species of *Chlorella* are perhaps the easiest among the algæ to isolate in pure culture, the process requiring little more than a direct application of bacteriological methods.

Attention should be called to another method—really a modification of the one just given—by means of which *Chlorella* species may be obtained in pure culture. Its application is not necessary in the species of *Chlorella* investigated, since

the vegetative cells can be so readily freed from adhering bacteria. But its general applicability to other forms justifies its mention at this place. *Chlorella*, like many other genera of the *Protococcales*, forms non-motile endogenous daughter cells which remain enclosed in the mother wall for varying lengths of time. The enclosed daughter cells are in all cases free from adhering bacteria. A group of daughter cells still enclosed within the mother membrane may be removed by means of a capillary pipette to a drop of sterile water, and from here to a succession of others until all readily removable bacteria have been left behind. The last transfer should be made to a drop of sterile water on a small sterile cover glass. By a slight pressure of a second cover glass, the mother membrane may be ruptured, liberating the enclosed, bacteria-free cells. The two cover glasses should then be introduced into a tube of liquid agar, the latter shaken vigorously, and finally poured into a Petri dish. Frequent isolations have been made in this way, and its importance in forms whose vegetative cells cannot be freed from adhering bacteria, and which do not form motile spores but only non-motile endogenous daughter cells, can hardly be overestimated.

Pleurococcus vulgaris Menegh.—The majority of *Pleurococcus* cells, when thoroughly washed, will be found free from bacteria. A difficulty which frequently arises is that the alga grows so very slowly that fungi—which are persistently present in *Pleurococcus* cultures—take entire possession of the plates before a transfer can be effected. But with careful searching, minute colonies—often consisting of but a few cells—can usually be found and successfully transferred. The transferred colony, however, usually makes extremely slow progress in its growth on agar. Much better results are obtained when transfers are made to evaporimeters (as devised by Livingston (15)) supplied with the mineral nutrient solution.

Scenedesmus sp., and *Kirchneriella sp.*—Both of these species were obtained in pure culture by washing and diluting clean, concentrated material in sterile mineral nutrient solution, and then plating. The great majority of the colonies of both species were contaminated with bacteria, pure colonies being very rarely found. This fact, together with the gelatin-

ous exterior characteristic of the cells of both species, makes it probable that the pure colonies developed, not from mature individuals, but from autocolonies (produced within mature cells) which either had just escaped from the mother cell or had done so during the vigorous shaking,—in either of which cases they are free from adhering bacteria.

Chlorococcum humicola (Näg.) Rabenh.—This species was isolated in the zoösporic condition. The alga, collected from soil, was placed in sterile mineral nutrient solution and after twenty-four hours produced zoöspores in abundance. Platings with these yielded numerous pure colonies from which successful transfers were made. In this connection it should be mentioned that all zoöspores thus far experimented with—including a considerable variety of forms—have been found free from bacteria. It is needless to say, therefore, that the presence of zoöspores in the life cycle of any alga provides a logical point of attack for its isolation in pure culture. While not all the attempts to isolate zoösporic forms in pure culture have proved successful, it is entirely probable that they will when the general technique is more closely adapted to individual forms.

Protosiphon botryoides (Kütz.) Klebs.—The vegetative plant of *Protosiphon*, with its root-like process extending into the soil and the large aerial portion, is so persistently covered with bacteria that its isolation in pure culture in this condition is quite impossible. With slight desiccation, however, large numbers of chlamydospores with dry non-gelatinous membranes appear, which, at least so long as they remain enclosed within the mother membrane, are free from bacteria. From these, isolations in pure culture can be readily made according to the second method suggested for *Chlorella*—by carefully washing an individual plant filled with chlamydospores, liberating the latter by teasing with needles or by a slight pressure of the cover glass, and plating in the usual manner. Another method which has yielded pure cultures, but which is not to be recommended because it is far less reliable than the one just described, is based on the use of the motile gametes. When vigorous *Protosiphon* plants, growing on soil, are covered with distilled water, gametes, which congregate in the lighted

side of the vessel, are produced in large numbers. Plates made with this material yield an occasional pure culture, but most of the gametes fail to develop. It is impossible at present to say whether the colonies develop from newly formed zygotes or from gametes which fail to conjugate.

Stigeoclonium tenue (Ag.) Kützing.—The ease and certainty with which zoöspores can be induced to develop in this form, and their extreme abundance, makes it, although a filamentous alga, an especially easy one to isolate. Freshly collected and thoroughly washed filaments of *Stigeoclonium*, placed in distilled water or sterile nutrient solution, will, in from twelve to twenty-four hours, develop a great abundance of zoöspores. Cultures prepared in this way contain so small a number of bacteria that plates containing a hundred or more *Stigeoclonium* zoöspores are sufficiently free from bacterial colonies to render numerous successful pure transfers possible. Although a filamentous form, *Stigeoclonium* grows exceedingly well on the mineral nutrient agar. While other members of the *Chætophoraceæ* were not experimented with, it is reasonably certain that forms like *Microthamnion*, *Chætophora*, and *Draparnaldia*, all of which readily yield large quantities of zoöspores, may be obtained in pure culture by a method identical with or similar to the one employed in the isolation of *Stigeoclonium*.

Oedogonium sp., and *Vaucheria sp.*—While neither of these forms were obtained in pure culture, the observations made render it altogether likely that this will be possible when a little more attention is given to the cultural solutions. Repeated trials with the vegetative filaments demonstrated that from the latter no pure cultures could be obtained directly. The oöspore proved equally unsatisfactory because the oögonial wall is covered with adhering bacteria. Again, the oöspore is, in most cases, so firmly and completely united with the oögonial wall that its separation from the latter is at present impossible. In both forms, however, zoöspores are readily obtained, and preliminary experiments demonstrated that these, like zoöspores in general, are bacteria-free. Where zoöspores could not be obtained in large quantities, individual ones were isolated with sterile pipettes, washed repeatedly in sterile water, and then either plated in the usual manner, or introduced into a

tube of sterile mineral nutrient solution. Although the great majority of such isolations remained bacteria-free, the zoöspores failed to develop, and finally died. It is only necessary, therefore, to find some medium in or on which the zoöspores will germinate and develop into plants, to effect a pure culture of *Vaucheria* or *Oedogonium*. *Bulbochæte* was not used, but in all probability this form will lend itself to a similar technique.

Conjugales.—Thus far it has not been possible to obtain a pure culture of any member of the *Conjugales*. The representatives of this order, in their vegetative phases, are provided throughout with an exterior gelatinous investment which is very generally impregnated with bacteria. All attempts to obtain pure cultures from vegetative individuals failed. Further, there is a complete absence in the order of motile spores and, in general also, of separable, asexual, endogenous spores. The zygospore, therefore, suggests itself as a possible means of solving the problem, especially in those forms where it is produced endogenously, and where it does not subsequently coalesce with the wall of the gametangium. While pure cultures were not obtained from these, the method used in *Spirogyra setiformis* is of interest and may prove serviceable in the ultimate isolation of these forms in pure culture.

Filaments containing mature zygospores, but in which the zygospore-containing cell walls were still completely intact, were washed repeatedly in sterile water and then broken up as thoroughly as possible with needles; in this process, numerous zygospores were freed from the enclosing walls, later to be taken up with sterile pipettes, and transferred to sterile drops of water. Each zygospore was subsequently transferred from ten to twenty times to fresh, sterile water drops, and finally taken up with a sterile pipette. When a considerable number of zygospores had thus been isolated, they were introduced into a tube containing a few cc. of sterile water, vigorously shaken, and the entire contents poured out into a Petri dish containing a layer of sterile nutrient agar. After rocking the dish for a short time, it was allowed to remain quiet until the zygospores had settled down on the surface of the agar. The free water was then very slowly and carefully, but completely, drained from the surface of the agar, and the plate allowed to remain

in the light. While in a few cases bacterial colonies developed about the zygopsores, it was found that the great majority were free from all adhering bacteria. Such zygospores as were bacteria free were then transferred to test tubes containing sterilized mud and pond water. Although about sixty such transfers were made, not a single one yielded a growing culture, although zygospores kept in battery jars in the laboratory showed a high percentage of germination. It will require further experiments to find a suitable medium for the germination and subsequent growth of isolated zygospores. However, the isolation of bacteria-free zygospores justifies the opinion that with them it will, sooner or later, be possible to culture *Spirogyra* in a state of purity.

HETEROKONTÆ

Botrydium granulatum (L.) Greville.—This form is, in its general morphology, so similar to *Protosiphon*, that the technique, as regards the use of chlamydospores, described for the latter, is entirely applicable here. *Botrydium* when submerged, however, forms an abundance of zoöspores instead of gametes, and from these pure cultures can be obtained with great ease when plated in the usual manner. The method for using the chlamydospores can also be considerably abbreviated in *Botrydium*. When the plants form chlamydospores, the aerial globular portion of the plant collapses. The cell, however, is so large that the aerial bag can be torn open with fine sterile forceps, the spores removed under a hand lens with a needle and transferred directly to liquid agar. Platings made in this way show a very slight bacterial contamination, and pure transfers can be made in abundance. While a direct, bacteria-free transfer has not been thus effected, it is altogether probable that it can be done. The pure transfers of *Botrydium* having been obtained, it was found that their development on agar was extremely slow, and ultimately all of the cultures died. Further experiments will be necessary in order to provide a favorable medium for growth. The clay-cup evaporimeter may perhaps prove of service in this connection as it did in the case of *Pleurococcus*.

Botrydiopsis sp.—This form was found abundantly during

one season on soil in the greenhouses. The vegetative cells when placed in water readily produce zoöspores, and isolations were made from these with little difficulty. Unlike *Botrydium*, this form grows exceedingly well on the mineral nutrient agar.

BACILLARIALES

The diatoms were encountered only incidentally in connection with other forms, and no particular effort was made to isolate forms in pure culture. Although diatoms, in general, have a gelatinous exterior, a small *Navicula* was on several occasions obtained in pure culture and grown successfully. It should be said, however, that the great majority of diatom colonies obtained were contaminated with bacteria.

CYANOPHYCEÆ

In the class *Cyanophyceæ*, the most difficult problems of isolation are met. The almost universal presence of an abundance of external mucilaginous material, the complete absence of ciliated reproductive cells, and the virtually complete absence of free, endogenous spores, renders the technique particularly difficult. The gelatinous investments are, in all cases investigated, impregnated with bacteria which cannot be completely removed by the most vigorous washing. Among the forms studied were *Aphanocapsa*, several species each of *Oscillatoria*, *Nostoc*, and *Anabæna*, *Cylindrospermum*, and *Microcoleus*. Of these, only two species, one of *Oscillatoria* and one of *Microcoleus*, were obtained in pure culture.

In the isolation of these two forms, silicic acid jelly was found to be indispensable. While directions for preparing this medium are to be found in many places in the literature, certain difficulties encountered in its preparation have made it desirable to give at this time, and in some detail, the method used.

As regards the preparation and mixing of the sodium silicate and hydrochloric acid solutions, the directions given by Smith (22) may be followed. It is only necessary to point out in this connection that if Merck's "sodium silicate pure crystals" is used, the solution should be made up with cold water. If hot water is used, an unidentified substance (insoluble in cold water) goes into solution, and frequently causes the coagulation

of the silicic acid-hydrochloric acid mixture before dialysis is complete. A point of very great importance is the preparation of the collodion dialyzing bags. As has been pointed out by Kellerman (12), and others, the degree of permeability of the bags depends, in a large degree, upon the way in which they are made. If the guncotton solvent is made from equal parts of ether and absolute alcohol, the bags will, in most cases, have a very low permeability, and coagulation of the enclosed silicic acid solution will frequently result before dialysis is complete. The degree of impermeability is further increased by drying the bags rapidly. If, however, 95 per cent (instead of absolute) alcohol is used, and the bags are allowed to dry spontaneously by inverting the test-tubes in which the bags are being prepared in suspended wire baskets, a much higher degree of permeability will be obtained.

Bags prepared with 95 per cent alcohol were used, and the silicic acid-hydrochloric acid mixture dialyzed in tap water until the chloride content was no greater than that of the water. The silicic acid solution was further purified by dialyzing in changes of ordinary distilled water and finally in triply distilled, nitrogen-free water. In this extended dialysis, a considerable portion of the silicic acid is lost, and it usually becomes necessary to concentrate the solution to obtain a jelly of sufficient firmness. This is best carried out in heavy, two-liter suction-flasks in which the pressure is reduced until the solution boils at from 35 to 40°C. If the concentration is carried out at higher temperatures, coagulation sometimes results. In order to prevent the violent bumping which always takes place unless some special precautions are taken, it is only necessary to bring through the rubber stopper at the top of the suction-flask a glass tube drawn out at the bottom to a very fine capillary, which dips into the solution. The top of this tube, outside of the rubber stopper, should be provided with a piece of rubber tubing and pinch cock to regulate the intake of air. The air thus admitted may first be washed to remove carbon dioxide, ammonia, or other impurities. The concentration should be continued until a sample, when congealed, has the proper consistency. The directions given by Smith (22) for coagulating the medium apply here and it need only be mentioned

that the concentration of the mineral nutrients employed in the agar, 0.1 per cent, is quite sufficient to bring about coagulation.

After it had become probable that no blue-green alga, in the ordinary vegetative condition, could be isolated by the usual plating method, tubes containing from two to three inches of solid, sterile, synthetic agar were inoculated at the surface with a species of *Oscillatoria*. The tubes were then completely wrapped in black paper, leaving only the very bottom exposed to the light, and inverted. It was hoped that in the rapid growth of the alga through the agar, the bacteria might be left behind. The growth toward the light in some cases amounted to eight mm., and more, per day. When the growth had approximately reached the bottom of the tube, the end of the latter was broken away, the surface of the agar seared, and transfers made from the interior of the agar plug. Although the experiment was repeated many times, and a total of at least fifty transfers made, a pure culture was never obtained, bacteria always being present. Large Petri dishes, containing a layer of sterile synthetic agar, were then inoculated at one edge with a species of *Oscillatoria*, and the dishes so placed that the point of inoculation was farthest away from the light. The alga grew rapidly (on the surface of the agar) toward the light, and just before reaching the opposite edge of the dish, transfers were made from the farthest advanced filaments. Although transfers to fresh agar surfaces were continued to the number of six, a pure culture was never obtained.

The experiment was then repeated, surfaces of silicic acid jelly replacing those of agar, with the result that numerous pure transfers were obtained from the second plate. A species of *Microcoleus* was obtained in pure culture in an identical manner.

Most members of the *Oscillatoriaceæ* are provided with a sharply delimited, gelatinous sheath. Reproduction is effected by the formation of hormogonia which glide out of the sheath, move about slowly for a time, and then come to rest. In forms like *Microcoleus*, *Lyngbya*, and some species of *Oscillatoria* in which the hormogonia escape from definite sheaths,

leaving the latter behind, it is fairly certain that the hormogonium is originally free from bacteria, but becomes contaminated in passing through the older portion of the empty sheath and out of its terminal opening, both of which are more or less infected with bacteria. The persistence with which the bacteria cling to the hormogonium of *Oscillatoria*, once having infected it, is clearly shown by cultures on agar surfaces. Although a single hormogonium may have moved as much as two inches away from its parent filament, creeping all the while over a sterile agar surface, the hormogonium will be found covered with bacteria, and the path over which it moved will be clearly indicated by a continuous, linear colony of bacteria. With the use of silicic acid jelly, however, the multiplication of the bacteria is reduced to such an extent that, after a time, hormogonia escape uncontaminated, and begin the development of pure colonies. Transfers from these, however, grow very slowly and in most cases eventually die. It seems probable, when *Oscillatoria* and *Microcoleus* have been completely separated from the invariably present bacteria, that the media which were favorable in the presence of the bacteria, become unfavorable in their absence. Further work will be necessary to grow these forms successfully after they have been isolated in pure form. The silicic acid jelly method was also attempted with the above mentioned heterocystic forms; however, up to the present time, no successful isolations have been made.

DISCUSSION

It is apparent that the technique involved in the isolations just referred to depends entirely on mechanical separation of one kind or another. This method is reasonably efficient in those species in which zoöspores or other free endogenous spores are readily obtainable, or in which vegetative cells are either free from bacteria or can be rendered so by mechanical means. It is true that even in some species forming free endogenous spores, the above methods have not yielded pure cultures, as, for instance, in *Vaucheria*, *Oedogonium*, and *Spirogyra*. In these cases, however, it should be pointed out that it is not the isolation technique which is at fault but rather

the cultural methods. Zoöspores and zygospores, respectively, free from other organisms, were obtained in these cases but failed to develop in the cultural media subsequently supplied. There can be little doubt, however, that the latter difficulty will be overcome in time.

Except in the *Oscillatoriaceæ*, little progress was made in the *Cyanophyceæ*. The problem appears especially difficult in the *Coccogoneales* where all forms of motile reproductive bodies are absent, and in which the vegetative cells apparently cannot be rendered free from adhering organisms by mechanlica means. Even in the heterocystic *Hormogoneales*, the situation is a difficult one, the more slowly moving hormogonia apparently being unable to escape the bacteria.[1] While no experiments were made along these lines, it appears highly desirable to attack the problem in the latter group through the spore. It is well known that the spores of blue-green algæ are extremely resistant to heat, and it does not appear improbable that the bacteria—especially if they are all in the vegetative condition—could be killed by heat, leaving the algal spores unharmed. Chemical sterilizing agents may also prove of value here. The latter may also prove serviceable with members of the *Coccogoneales* and certain of the grass-green algæ which have thus far failed to yield to the technique employed.

Conclusions

1. By adapting methods of pure culture technique to individual species of algæ, it has been possible to isolate in pure culture the following forms:

Chlorophyceæ.—Chlamydomonas pisiformis Dill forma *minor* Spargo, *Stichococcus bacillaris* Näg., *S. subtilis* (Kütz.) Klercker, *Ulothrix sp.*, *Chlorella vulgaris* Bey., *Chlorella sp.*, *Pleurococcus vulgaris*, *Scenedesmus sp.*, *Kirchneriella sp.*, *Chlorococcum humicola* (Näg.) Rabenh., *Protosiphon botryoides* (Kütz.) Klebs, *Stigeoclonium tenue* (Ag.) Kützing, and a number of others of uncertain identity.

[1] In a contribution which has just appeared (Kulturversuche mit Chlorophyll-führenden Mikroorganismen, III. Zur Physiologie der Schizophyceen. Beitr. z. Biol. d. Pflanzen 12: 49–108. 1913), Ernest G. Pringsheim reports the isolation in pure culture of a species of *Nostoc*. The method used was that of repeated transfers to sterile silicic acid jelly plates.

Heterokontæ.—Botrydium granulatum (L.) Greville, and *Botrydiopsis sp.*

Bacillariales.—Navicula sp.

Cyanophyceæ—Oscillatoria sp., and *Microcoleus sp.*

2. In addition, zoöspores from *Vaucheria* and *Oedogonium*, and zygospores from *Spirogyra* have been isolated free from other organisms.

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THE IDENTIFICATION OF THE MOST CHARACTERISTIC SALIVARY ORGANISM, AND ITS RELATION TO THE POLLUTION OF AIR[1]

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Introduction

Bacteriologists and sanitary engineers have, within the last score of years, given much attention to the detection of excremental pollution in water. They have shown that by making it possible to recognize certain characteristic accompanying organisms, bacteriological methods are capable of revealing this kind of pollution even when it exists to such a small degree as to be beyond the range of chemical detection. Small as these quantities of contaminating substances may seem, they may nevertheless endanger the health of a whole community by exposing it to possible pathogenic organisms derived from the excreta of a diseased host.

It is not merely by the aggregate bacterial yield that the potability of a water in its relationship to disease is judged, but more specifically by the species of bacteria present, and their relative abundance. The micro-organisms which serve as an index of pollution, and for which special quantitative examination is made, are the members of the colon group. These, from their constant presence and relative abundance, are characteristic of material of excremental origin. Their presence in water in sufficient quantity indicates pollution, and their relative abundance serves as an index to the extent of the latter.

Bacteriological technique has not as yet been applied to the same extent in the detection of pollution in air. Chemistry has, up to the present time, been of more practical value here.

[1] An investigation carried out at the Missouri Botanical Garden in the Graduate Laboratory of the Henry Shaw School of Botany of Washington University, and submitted as a thesis in partial fulfillment of the requirements for the degree of master of arts in the Henry Shaw School of Botany of Washington University.

The proportion of carbon dioxide is still the standard mainly relied on for estimating pollution of air by materials given off from the human body, although it is recognized that other factors may be of more importance. This method of examining air, however, is of little or no value in furnishing an index to the probable or possible contamination with disease-producing germs, for there is at present no reason for believing that such organisms are given off in the breath during ordinary quiet breathing. Thus, M. H. Gordon[1] calls attention to the following: Tyndall observed that expired air is optically purer than inspired air; Cornet found air expired by tubercular patients to be free from the tubercle bacillus; and Straus has shown that expired air is not only comparatively free from bacteria, but that it is considerably purer in this respect that inspired air. It nevertheless appears probable that bacteriology rather than chemistry will furnish a means of investigating the pollution of air by disease-producing germs. The problem at hand is to devise, if possible, a method for estimating the degree of pollution of air by pathogenic organisms (given off from the human body) in a manner similar to that employed in estimating the extent of pollution of water by similar organisms of excremental origin.

HISTORICAL

It appears that the present status of bacteriological analysis of air is comparable to that of bacteriological analysis of water some years ago, when the total number of bacteria in a given quantity was the chief factor determined. There are various ways in which pathogenic organisms may gain access to the air and ultimately to another individual. In addition to transfer by direct contact, disease-producing organisms may be given off in the urine, in feces, in sputum, or from the surface of the skin. Recently, also, attention has been called to the possibility of the pollution of air by the scattering of fine particles of mucus and saliva from the mouth in the acts of coughing, sneezing, and loud speaking. The latter methods of air pollu-

[1] Report on a bacterial test for estimating pollution of air. Supplement to the Thirty-second Annual Report of the Local Government Board (London), containing the Report of the Medical Officer for 1902–3. 421–471. 1904.

tion are the ones to be considered in this investigation. They doubtlessly constitute an important means whereby pathogenic organisms enter the air from an infected person, subsequently to be transmitted to other individuals.

The discharge of sputum furnishes the most obvious way whereby pathogenic organisms may be expelled from the mouth. The expectorated mucus dries, and, in the form of dust, may later be inhaled to produce infection. The work of Flügge and members of his school,[1] however, has drawn attention to a more direct and no less important way by which germs may be aërially conveyed from the mouth. The problem of transmission of micro-organisms by means of particles of mucus expelled from the mouth in various expiratory acts, was attacked in two ways by the investigators referred to above: 1. The mouth was artificially infected with a culture of *Bacillus prodigiosus*. This organism was chosen because the red pigmentation of the colonies renders the identification easy. After agar plates had been placed at various distances from the person being experimented upon, the individual proceeded to speak, cough, sneeze, etc. At the end of the experiment the agar plates were covered and incubated at 25° C. for 3 days, during which time the characteristic red colonies of *B. prodigiosus* made their appearance. The possibility of error due to the previous presence of this organism in the air of the room was excluded by exposing a series of agar plates immediately before the experiment began, with the result that in all cases the organism failed to appear. The length of time that droplets of mucus remained suspended in the air after the several expiratory acts was determined by exposing plates at various periods after the experiment had been completed. 2. Glass slides or empty Petri dishes were placed at various distances from a tubercular patient. The droplets of mucus expelled during coughing, and deposited upon the glass slides, etc., were either examined microscopically or were washed off and injected intraperitoneally into guinea-pigs. In the former case a bacillus giving the characteristic staining reaction of the tubercle bacillus was found, and in the latter the development of tuber-

[1] Gordon, *loc. cit.*

culosis in the inoculated animals resulted. In other experiments, guinea-pigs, instead of being inoculated, were directly exposed to the coughing of tubercular patients with the result that a number of the animals so exposed contracted tuberculosis. Varied and repeated experiments along these lines established the fact that in the acts of coughing, sneezing, and loud speaking, fine droplets of mucus are ejected into the air, that they float about and may be wafted by air currents, such as obtain in ordinary rooms, to a distance of from 24 to 40 feet.

The most thorough investigation in recent years of the problem of air pollution with micro-organisms was made by Dr. M. H. Gordon.[1] This author believed that the positive recognition of disseminated saliva constituted an important step in the development of an applicable bacteriological method for the examination of air. By bacterial analyses of a number of samples of saliva obtained from normal individuals, Dr. Gordon determined that the streptococci are the organisms most abundantly present in saliva. Of these he was able to differentiate four morphologically different types—*longus*, *medius*, *brevis*, and *conglomeratus*. In endeavoring to differentiate these organisms on a physiological basis a study was made of their virulence, relation to oxygen, optimum growth temperature, pigment production, motility, gelatin liquefying power, indol production, action on litmus milk at 37°C., and action on various carbohydrates.

It was found that the micro-organism which is most useful in the detection of droplets of saliva is *Streptococcus brevis* because it is the only one among the salivary cocci found which changes the color of neutral red broth to yellowish green, and produces acid and clot in milk. Having developed a means of differentiating the coccus most characteristic of saliva, Gordon next examined the open air for the presence of micro-organisms characteristic of saliva. In these experiments broth plates were exposed for a stated length of time and incubated anaërobically at 37°C. In but very few cases were the organisms isolated from the air.

A further means of differentiating the characteristic salivary

[1] *Loc. cit.*

coccus from the air cocci was sought in the action of the two on various organic substances. In this capacity the several broths containing lactose, syringin, and coniferin, proved especially serviceable. In lactose broth the typical salivary coccus was positive, i. e., it produced acid, whereas the air cocci were negative. In the syringin and coniferin broths, the air cocci were positive, the typical salivary coccus negative.

To determine whether or not particles of saliva were disseminated through the air during the acts of coughing, sneezing, and loud speaking, Gordon performed experiments in a large and in a small room, using, at first, Flügge's method of artificially infecting the mouth with a living culture of *Bacillus prodigiosus* and placing sterile agar plates at various distances in front of and behind the speaker. After ½–1 hour of loud speaking, it was found that *B. prodigiosus* had been disseminated to a distance of 40 feet in front of and of 12 feet behind the speaker. In other experiments in which no artificial infection of the mouth was resorted to, but in which the characteristic salivary coccus served as the index of dissemination, it was found that after ¼–1 hour of loud speaking *Streptococcus brevis* appeared on broth plates placed as many as 12 feet in front of and behind the speaker. In similar experiments in which speaking was continued for one hour in an ordinary conversational tone, no dissemination of the salivary *Streptococcus* could be detected.

From his experiments Dr. Gordon inferred that there were certain streptococci normally present in saliva which are applicable for the detection of droplets of saliva in air in much the same manner that *Bacillus coli* can be applied for the detection of fecal matter in water.

The Identification of the Most Characteristic Salivary Organism

With a view of determining the organism most characteristic of saliva, I have undertaken, as a first step, a bacteriological analysis of the saliva of a normal individual. In this examination special attention was paid to the type of organism most abundantly present. Having determined the type, i.e., whether bacillus, coccus, or spirillum, characteristic reactions for it were next sought in order to render its recognition easy. Since

a possible relation of the characteristic salivary organism to the pollution of air was to be investigated, it was necessary to examine the outdoor air free from human contamination for the presence of micro-organisms closely allied to those characteristic of saliva. As particles shed from the skin may be present in the air, it was further necessary to examine those micro-organisms found on the skin which were closely allied to the ones characteristic of saliva.

In examining the saliva for the type of micro-organism most constantly present, i.e., whether bacillus, coccus, or spirillum, the dilution method was used. It is reasonably safe to assume, after repeated trials, that the type of micro-organism which persists longest in continued dilutions is the type most abundant in the material examined. This is true provided the medium on which the organism is grown is approximately equally favorable for the development of all the types present. The dilutions were carried out as follows: A sample of saliva was collected in a sterile test-tube and 1 cc. introduced into a second tube containing 9 cc. of sterile distilled water. The contents of the latter were then thoroughly mixed and 1 cc. of the liquid introduced into a third tube likewise containing 9 cc. of sterile distilled water. This procedure was repeated until 6 dilutions had been effected. Obviously, 1 cc. quantities of each of the 6 successive dilutions contain respectively $\frac{1}{10}$, $\frac{1}{100}$, $\frac{1}{1,000}$, $\frac{1}{10,000}$, $\frac{1}{100,000}$, and $\frac{1}{1,000,000}$ cc. of saliva.

One plate each from dilutions 4, 5, and 6 was made, 1 cc. of the respective dilutions being introduced into 10cc. of nutrient + 1 agar. After thorough mixing, the plates were incubated aërobically for 24 hours at 37°C. The plate made from dilution 5 produced 20 colonies, whereas the one from dilution 6 showed no growth. From each of the 20 colonies a cover-glass preparation stained with gentian violet was made. Microscopic examination revealed the fact that each of the 20 colonies was composed of micro-organisms of the coccus type. Transfers were then made to agar slopes which were incubated at 37°C., for 24 hours. The cultures obtained in this manner were numbered from 1 to 20 and kept at 20°C., as stock cultures.

In examining the open air, sterile agar plates were exposed as indicated in table I.

TABLE I

DATA ON THE COLLECTION OF AIR COCCI

Place of exposure.	Time of exposure	Total colonies on plate after 24 hrs. at 37°C.	Coccus colonies on plate after 24 hrs. at 37°C.	Number given to stock culture	Remarks
Window sill outside of laboratory, 2nd floor.	15 minutes.	14	7	21 to 27 incl.	
On shelf, center of laboratory room.	do.	2	0		One person in room. Abundance of *Monilia* present.
On table, in reading room.	do.	2	0		do.
On table, in plating room of laboratory.	do.	0	0		*Monilia* suppressed growth.
On table, in basement	do.	3	0		
On lawn, in garden	do.	9	4	28 to 31 incl.	
In living room.	do.	7	6	32 to 37 incl.	
Window sill, 4th floor, downtown section	do.	18	0		Much soot on plate.
On table, in draughting room.	do.	1	1	38	One person in room.

After exposure the plates were covered and incubated aërobically for 24 hours at 37°C. Stained preparations of all colonies developing were made and examined under the microscope. The coccus forms were transferred to agar slopes, and after incubation for 24 hours at 37°C., were kept in stock at 20°C. Several of the plates exposed in various parts of the laboratory building were rendered worthless by an abundant growth of *Monilia sitophila*.

The method of examining the skin for organisms closely allied to those characteristic of saliva, was as follows: Test-

tubes, each containing 10 cc. of distilled water and a piece of linen 2 inches square, were sterilized in the autoclav for 15 minutes at 15 pounds pressure. Samples were taken from three parts of the body of a normal individual, namely, the calf of the leg, the thigh, and the chest. This was accomplished by briskly rubbing the portion of the body from which the sample was to be taken with the piece of linen held in sterilized forceps, and later replacing it in the tube of sterilized water. From these dilutions, after being thoroughly shaken, about ½ cc. quantities were plated in 10 cc. of nutrient agar. From each plate 2 coccus colonies were selected from which transfers were made to agar slopes. These, after 24 hours at 37°C., were kept as stock cultures at 20°C.

There were now in stock a total of 44 pure cultures of *Coccaceæ*, 20 from saliva, 18 from the open air, and 6 from the skin.

MORPHOLOGICAL CHARACTERS

The form of the individual cell is of little value in differentiating the species of *Coccaceæ*, for under conditions favorable to their growth, all appear as regular spheres. Irregular oval cells occur at times, but the form usually becomes normal after cultivation. Some writers lay considerable stress on the value of cell grouping in the *Coccaceæ* as a means of differentiation. With the utmost care in cultivation and staining, however, this could not be verified in the cultures under observation. All the cultures examined contained cells occurring singly, in pairs, in short chains, and in masses, but in no case did the cells of any specific culture exhibit a distinct tendency to occur in any one form. A stained cover-glass preparation showed various cell groupings in different parts of the same microscopic field.

Cell grouping was studied in the following manner: An oese of sterile +1 bouillon was placed on a sterile cover glass, inoculated with a 24-hour culture of the organism to be examined, and inverted on a Van Tieghem cell containing a few drops of sterile distilled water. After sealing the cover glass on the cell with vaseline, the preparation was incubated for 24 hours at 37°C. At the end of this time the cover glass was removed and the drop of water containing the organism allowed to

evaporate. Then, 3 drops of mercuric chloride solution were applied and after 2 minutes washed off with distilled water. Following this, the preparation was treated with a few drops of 1 per cent acetic acid for 5 minutes, again washed in water, and finally stained for about 15 seconds with a few drops of gentian violet. After washing, and drying in the incubator at 37° C., the vaseline was removed from the cover glass with xylene, and the preparation mounted in balsam and examined under the microscope. The relation to Gram stain was observed on 2 and 4-day agar cultures incubated at 20°C. The preparations were treated with aniline oil-gentian violet for 1½ minutes, with Gram's iodine solution for 1½ minutes, and finally with 95 per cent alcohol for 3 minutes. The reactions are recorded as "– –" (decolorized in both tests), "+ –" (stained in one test and decolorized in the other), and "+ +" (stained in both tests).

CULTURAL CHARACTERISTICS

All cultural characteristics were observed in streak cultures on agar slants after 14 days' incubation at 20°C., and 37°C. Such differences as developed between the cultures were almost entirely variations in color and vigor of surface growth. Under the latter, 5 types were distinguished as follows:

1. Growth very faint and veil-like, or forming scattered translucent colonies.
2. Growth better, but still meager.
3. Growth good, but not abundant.
4. Growth abundant.
5. Growth very heavy.

In the study of chromogenesis, apparent differences in pigment production, due to unequal vigor of growth or evaporation, were, so far as possible, eliminated. This was accomplished by examining in each case the same amount of material—a loopfull—spread evenly on white drawing paper having a rough surface. After drying at room temperature, the color of the pigment produced was compared with the colors as given by Ridgway[1].

[1] Color standards and nomenclature. 1912. [Published by the author, Washington, D. C.]

This author uses as a basis the solar spectrum with its six fundamental colors and intermediate hues, augmented by a series between violet and red not in the spectrum.

BIOCHEMICAL REACTIONS

The production of indol was investigated in 5-day peptone broth cultures incubated at 37°C. One cc. of a 10 per cent sulphuric acid solution was thoroughly mixed with the broth culture, and then 1 cc. of a freshly prepared 0.01 per cent solution of sodium nitrite was carefully run in on top of the mixture. The appearance of a pink ring at the juncture of the nitrite solution with the acid-peptone solution, was regarded as an indication of the presence of indol. A blank determination for purposes of comparison was made in each case. The action on neutral red broth as regards change in color was observed in cultures incubated for 5 days at 37°C., in the presence of hydrogen.

The organisms were further grown in solutions of nitrate broth to determine whether or not reduction takes place, and if so, whether to nitrite or to ammonia. In carrying out the test a tube of nitrate broth was inoculated with the organism to be tested, and incubated for 4 days at 37°C., an uninoculated tube of nitrate broth being similarly treated to serve as a check. At the end of 4 days, 3 cc. of the broth were removed to a clean test-tube, and 2 cc. each of a naphthylamine solution and of a sulphanilic acid solution added. The development of a red color indicates the presence of nitrites, the intensity of the color being proportional to the amount of nitrites present in solution. To test for ammonia in the remaining portion of the culture, a few drops of Nessler's solution were added. The appearance of a yellow color or precipitate indicates the presence of ammonia. In studying the liquefaction of gelatin by the cocci under observation, the extent of the action only was determined. This was accomplished by spreading a suspension of the organism over the surface of gelatin in 10 mm. tubes. It was found that the amount of material used in this inoculation did not affect the total amount of liquefaction, i.e., whether the amount of transferred material was large or

small the extent of the liquefaction after 30 days' growth at 20°C., was the same with any one organism.

In the study of the action on sterile certified milk particular attention was paid to the coagulation of the milk and to the production of acid. Observations were further made on the effect of the organisms on lactose, saccharose, mannite, salicin, inulin, sorbite, raffinose, and rhamnose. The medium in which these organic substances were used was prepared according to Dr. Houston's formula, as follows:

Liebig's beef extract	1.0 per cent
Peptone	1.0 per cent
Organic compound to be tested	1.0 per cent
Sodium bicarbonate	0.1 per cent
10 per cent litmus solution	1.0 per cent

The medium, neutral in reaction to litmus, was sterilized for 15 minutes at 15 pounds pressure in 500 cc. containers, from which sterile fermentation tubes, provided with glass caps, were directly filled. In doing this it was necessary to take utmost precautions to obviate any possibility of contamination. The various organic media thus prepared were inoculated, not from an agar slope, but from a 48-hour broth culture. Gas formation and the production of acid in the several media were observed after 3 days' incubation at 37°C.

DISCUSSION OF RESULTS

A thorough study of the results will now be made with a view of finding, if possible, some characteristic or group of characteristics, morphological or biochemical, which may be used in differentiating the salivary cocci from the coccus forms of the air and the skin.

The cell grouping varies throughout, there being no arrangement characteristic of any particular group. As observed, all the forms occur in groups, chains, and pairs. As regards the deportment of the various organisms toward the Gram stain, it was noted that all of the salivary cocci gave a positive reaction in both tests; of those from the air, 3 were positive in the two tests, 8 alternately negative and positive, and 6 negative throughout; of the skin cocci, 4 were positive and 2 negative

in both tests. This stain, as may readily be seen, is of no differential value here, for, although the salivary cocci react positively throughout, both positive and negative reactions occur among the air and skin forms.

The production of indol among coccus forms is very uncommon. Of the salivary cocci under observation, none produced indol, and of the air and skin forms only one from each group produced it. The change of color in neutral red broth is, apparently, more frequently brought about by the salivary cocci than by the air and skin forms, but this difference is not sufficiently well marked to be of differential value. Of the 20 salivary cocci, 12 produced fluorescence, whereas only 1 of the air and none of the dermal forms produced this change. All of the forms under observation reduced nitrates to ammonia. Of the salivary forms, 14 out of 20; of the air cocci, 5 out of 18; and of the skin cocci, 5 out of 6, reduced nitrates to nitrites. It thus appears that the reduction of nitrates to ammonia is very common among members of the *Coccaceæ*, but that the reduction to nitrites only is variable and not characteristic of any one type.

The average amounts of gelatin liquefied after 30 days' growth at 20°C., are as follows: by the salivary cocci, 2.8 cc.; by the air forms, 1.9 cc.; and by those of the skin, 1.4 cc. Fifteen out of 20 of the salivary organisms, 15 out of 18 of the air forms, and 4 out of 6 of the skin cocci, liquefied gelatin. Summing up the results obtained from the experiments on gelatin liquefaction, it is to be noted that, in general, the salivary cocci liquefy gelatin more readily than do the air or skin forms, but aside from this it is apparent that there is nothing to warrant the use of gelatin as a differential medium.

The results of the experiments on vigor of surface growth on agar slopes at 20°C., and 37°C., are given in table II. While it may be said, in general,—from the results given in this table—that the salivary cocci grow somewhat more vigorously at 37°C. than at 20°C., the air forms better at 20°C. than at 37°C., and the skin organisms about equally well at the two temperatures, the differences are not sufficiently pronounced to impart to the factor of vigor of surface growth any marked value as a differential characteristic.

TABLE II

DATA ON THE VIGOR OF SURFACE GROWTH OF AIR, SALIVARY, AND DERMAL COCCI

Source of organism	Temperature of incubation	No. of cultures used	Growth characteristics					
			No growth	Very faint	Meager	Good	Abundant	Very heavy
Saliva	20°C.	20	—	3	7	10	—	—
Air	20°C.	18	1	—	—	4	7	6
Skin	20°C.	6	—	1	2	2	1	—
Saliva	37°C.	20	—	—	7	13	—	—
Air	37°C.	18	2	1	9	6	—	—
Skin	37°C.	6	—	1	—	4	1	—

In the following enumeration are listed the colors of the various pigments produced by the air, skin, and salivary cocci, the figure on the left having reference to the number in Ridgway corresponding to the particular pigment produced:

Salivary cocci

15″ d	Light pinkish cinnamon	3	15
15″ c	Intermediate between light pinkish and pinkish cinnamon	4	
15″ b	Pinkish cinnamon	4	
15″ a	Intermediate between pinkish cinnamon and cinnamon	3	
15″	Cinnamon	1	
21′ e	Intermediate between massicot and straw yellow	1	
23′ f	Naphthalene yellow	1	
19 f	Maize yellow	1	
19′ b	Mustard yellow	1	

One gave too little growth for determination of the color.

Air cocci

21′	f	Massicot yellow	4	16
21′	e	Intermediate between massicot and straw yellow	2	
21′	d	Straw yellow	1	
21′	b	Amber yellow	1	
19′	d	Naples yellow	1	
19′	b	Mustard yellow	4	
19′		Primuline yellow	1	
19	f	Maize yellow	1	
19	d	Buff yellow	1	
3′	b	Light Jasper red	1	

One form did not grow.

Skin cocci

19	f	Maize yellow	1	5
19	d	Buff yellow	1	
19	b	Apricot yellow	2	
21′	e	Intermediate between massicot and straw yellow	1	
		White	1	

At first glance the color of the pigments produced by the organisms would seem to furnish one mode of differentiation. In the majority of cases the salivary cocci produced cinnamon colored pigments, whereas pigments of a yellow color were usually produced by the air and skin forms. Closer inspection shows, however, that some of the salivary cocci, as well as the air forms, produce a maize yellow and a mustard yellow pigment; also that a maize yellow pigment and one intermediate between massicot and straw yellow are produced by representatives of both the salivary and skin cocci. It is apparent that these intergradations make the factor of pigment production largely inapplicable as a differential test.

In milk the salivary cocci with one exception produced acid and coagulated the medium, whereas none of the air forms and but one of the skin cocci gave this combined reaction. This attaches to milk considerable value as a differential medium. In the media containing the various organic substances

—sugars, etc., none of the coccus forms produced gas. All but one of the salivary cocci produced acid in the lactose medium, whereas none of the air cocci and but one of the skin forms deported themselves in this manner. This marks lactose broth as another medium of differential value.

The salivary cocci with but one exception produced acid in saccharose, the single exception being the organism which produced no acid in the lactose medium. Two air cocci and one skin form also produced acid in saccharose, but notwithstanding these exceptions, it appears that saccharose is a third valuable differential medium. In the mannite, salicin, inulin, sorbite, raffinose, and rhamnose broths none of the organisms produced acid, thus marking these organic substances as of no value in differentiating the types of cocci under investigation.

SUMMARY

In reviewing the preceding discussion of results we find three media, namely, lactose and saccharose broths, and milk, which are of value in differentiating the cocci most characteristic of saliva from those of the air and the skin. One of the salivary coccus forms did not produce acid in lactose and saccharose broths and formed neither acid nor clot in milk. This may have been, and probably was, an air or skin form. Among the air cocci are two which vary somewhat from the remaining air and skin forms in that they produce acid in saccharose broth. Neither of them, however, produces acid in lactose broth, nor acid or clot in milk and in these respects they differ markedly from the characteristic salivary forms. Of the skin cocci one gave the characteristic reactions of the salivary organisms, and it is not at all unlikely that this was a salivary coccus. In general, then, it appears that the organism most characteristic of saliva is a coccus form which produces acid in lactose and in saccharose broths, and acid and clot in milk.

FURTHER TESTS

To further test the validity of the reactions above referred to as furnishing a reliable means of differentiating between salivary cocci and those of other origin, two additional samples of saliva, from two different individuals, were examined,—

one from a middle aged white person (A), the other from a colored person (B).

The samples were collected and treated in a manner similar to that outlined in the early part of this paper. In the first case (A), transfers were made from all colonies on two plates, representing a dilution of one part saliva in ten billion. These subcultures, all of cocci, were numbered from 1 to 17 inclusive.

In the second case (B), transfers were made from 36 colonies which developed on one-third of a plate representing a dilution of one part saliva in ten billion. The entire series of cultures, numbered from 1 to 36 inclusive, although made from 36 colonies from a plate containing a total of 100 colonies, were found to be made up of coccus organisms. After being incubated in + 1 nutrient broth for 2 days at 37°C., each of the cultures from samples (A) and (B) was transferred to the three differential media,—lactose and saccharose broths, and milk. The results recorded in tables III and IV were observed after 3 days' incubation at 37°C. No gas was produced in any of the sugar media. A blank determination gave negative results throughout on the three media.

TABLE III

REACTIONS OF SALIVARY COCCI (A)

No. of culture	Lactose broth	Sacch. broth	Milk		No. of culture	Lactose broth	Sacch. broth	Milk		No. of culture	Lactose broth	Sacch. broth	Milk	
			Acid	Clot				Acid	Clot				Acid	Clot
1	+	+	+	+	7	+	+	+	+	13	+	+	+	+
2	+	+	+	+	8	+	+	+	+	14	+	+	+	+
3	+	+	+	+	9	+	+	+	+	15	+	+	+	+
4	O	O	O	O	10	+	+	+	+	16	+	+	+	+
5	+	+	+	+	11	+	+	+	+	17	+	+	+	+
6	+	+	+	+	12	+	+	+	+					

+indicates positive reaction. O indicates negative reaction.

From the above table it is evident that all but one of the coccus forms in series (A) produced acid in lactose and saccharose broths, and acid and consequent clotting in milk. The one exception was probably an air coccus.

TABLE IV

REACTIONS OF SALIVARY COCCI (B)

No. of culture	Lactose broth	Sacch. broth	Milk		No. of culture	Lactose broth	Sacch. broth	Milk		No. of culture	Lactose broth	Sacch. broth	Milk	
			Acid	Clot				Acid	Clot				Acid	Clot
1	+	+	+	+	13	+	+	+	+	25	+	+	+	+
2	O	+	O	O	14	+	+	+	+	26	+	+	+	+
3	+	+	+	+	15	+	+	+	+	27	+	+	+	+
4	+	+	+	+	16	+	+	+	+	28	+	+	+	+
5	+	+	+	+	17	+	+	+	+	29	+	+	+	+
6	+	+	+	+	18	O	+	O	O	30	+	+	+	+
7	+	+	+	+	19	+	+	+	+	31	+	+	+	+
8	+	+	+	+	20	+	+	+	+	32	+	+	+	+
9	O	+	O	O	21	O	+	O	O	33	+	+	+	+
10	+	+	+	+	22	+	+	+	+	34	+	+	+	+
11	+	+	+	+	23	+	+	+	+	35	+	+	+	+
12	+	+	+	+	24	+	+	+	+	36	+	+	+	+

+indicates positive reaction. O indicates negative reaction.

In series (B), 32 out of the 36 cocci reacted positively throughout on the three differential media. The remainder were positive with saccharose, but negative with lactose and milk, agreeing in this respect with the two air cocci to which reference has been made.

The reactions of the organisms from saliva (A) and (B)

further indicate that the production of acid in lactose and saccharose broths, and a similar production, together with clot, in milk, are characteristic reactions of the salivary cocci.

CONCLUSIONS

From the results of the preceding experiments it appears that a method applicable for the detection of the organisms characteristic of human saliva has been developed.

It must be acknowledged that the number of organisms examined is comparatively small, especially where those of the air and the skin are concerned. An absolute test of the validity of the adopted mode of identification would necessitate the examination of many hundreds of strains of cocci from numerous sources.

Nevertheless, the characteristic reactions of the salivary cocci examined seem to be sufficiently definite to warrant the assumption that the most characteristic salivary organism is a coccus form which produces acid in lactose and saccharose broths, and acid and clot in milk.

THE RELATION OF THE MOST CHARACTERISTIC SALIVARY ORGANISM TO THE POLLUTION OF AIR

Having identified the most characteristic salivary organism, the next problem is to isolate it from the air. Its frequency of occurrence must also be determined, as this often serves as an index to the degree of pollution. The isolation of the organism and the determination of its frequency of occurrence can be accomplished simultaneously.

Then come the problems (1) of devising an air-collecting apparatus suitable for all occasions, and (2) of determining the quantity of air to be examined and the terms by which the sanitary quality of the air shall be expressed.

In searching for a means of expressing the sanitary quality of air, let us consider the manner in which this is accomplished in drinking water. Authorities differ markedly on this subject. Shall a water be considered safe or unsafe for drinking purposes if *B. coli* is present in a 100 cc. sample, or shall its presence or absence in 10 cc. or 1 cc. samples be taken as the basis for the

classification? In lieu of a definite standard let us assume the following table[1]:

TABLE V

PRESUMPTIVE TEST FOR *B. COLI* IN WATER

Sanitary quality	cc. 0.01	cc. 0.1	cc. 1.0	cc. 10.0	cc. 100
Safe	O	O	O	O	+
Reasonably safe	O	O	O	+	+
Questionable	O	O	+	+	+
Probably unsafe	O	+	+	+	+
Unsafe	+	+	+	+	+

+ indicates positive presumptive test for *B. coli*.

We shall now endeavor to prepare a similar table for the purity of air, expressed in the number of salivary cocci present in given volumes. In the normal life processes, the volume of air inhaled is obviously much greater than the volume of water consumed, and this fact must be taken into consideration in establishing a criterion for the bacteriological examination of air. It has been estimated that the tidal air, i.e., the air taken in with each inspiration and given out with each expiration, amounts, in a normal adult when at rest, to one-half liter. Assuming the average frequency of respiration to be 15 per minute, the amount of air inhaled in one minute is 7½ liters, in one hour, 450 liters, and in one day, at least 10,000 liters. Taking the average amount of unboiled water drunk in a day as 2 liters, it would appear that 5,000 times as much air as water is required daily. Hence, the following table, based on table v, may be used to express the sanitary quality of air:

[1] Whipple, G. C. On the practical value of presumptive tests for *B. coli* in water. Techn. Quart. 16:18 e. m. 31. 1903.

TABLE VI

TEST FOR CHARACTERISTIC SALIVARY COCCI IN AIR

Sanitary quality	cc. 50	cc. 500	cc. 5,000	cc. 50,000	cc. 500,000
Safe	0	0	0	0	+
Reasonably safe	0	0	0	+	+
Questionable	0	0	+	+	+
Probably unsafe	0	+	+	+	+
Unsafe	+	+	+	+	+

+ indicates positive reaction in the three differential media adopted.

APPARATUS AND TECHNIQUE

As it was the intention to collect samples of air in places other than the laboratory, a portable apparatus was necessary. As devised, it consists essentially of a sand filter, a support for same with an attachment for alternately opening and closing the exhaust and suction, and a bulb, having a capacity of 16 oz., with the required amount of rubber tubing. (See plate 2.) The sand filter is of the standard type. It consists of a glass tube 100 mm. long and 10 mm. in diameter, fitted with a one-hole rubber stopper, through which passes a piece of 6 mm. glass tubing. This stopper, with its tubing, forms the support for a circular disc of bolting cloth with a 10 mm. layer of very fine clean quartz sand that passes through a 100, and is retained on a 140 mesh sieve.

The support consists of a rectangular piece of wood 12 x 1 x $\frac{3}{4}$ inches, fitted with a double pinch cock arrangement. Clamps for holding the filter in position are also provided. The rubber bulb is connected to the apparatus in such a manner that when pressure is applied to the former and the pinch cock opened, the air contained in the bulb is expelled through the exhaust without disturbing the sand in the filter in any way. This operation occupies but a few seconds of time. Upon releasing the pinch cock, and immediately thereafter the bulb, the air is drawn through the sand.

The volume of air exhausted from the bulb at each pressure was determined as follows: The bulb, filled with water, was weighed. Pressure was then applied, forcing out the water, after which the bulb was again weighed. The difference in weight in grams is approximately the volume of air in cc. exhausted by a similar pressure. In the calibrations the results varied but slightly. By placing the fingers on the bulb in a certain fixed position each time, it was found that the bulb could be made to deliver 300 cc. of air at each exhaustion and, consequently, to receive 300 cc. of air at each release of pressure. It was, of course, necessary to have all joints air-tight, this being accomplished by making all connections with rubber tubing and glass and using plenty of overlap.

The sand filter, after being plugged at both ends with cotton, was sterilized for 30 minutes at 15 pounds pressure. The rubber stopper support was allowed to fit very loosely into the tube during sterilization in order to prevent setting of the rubber. After the apparatus was removed from the autoclav, the stopper was immediately fitted in tightly, thus rendering the connection air-tight. The sand filter was always used within 24 hours after sterilization. It was connected to the support as shown in plate 2.

When operated in public buildings or conveyances, the support, with the filter, was wrapped in stiff paper in such a manner as to permit of the easy operation of the pinch cock and exhaust. The apparatus thus wrapped was held in the left hand and from it heavy rubber tubing passed down the left coat sleeve and then diagonally across to the right coat pocket where it was connected to the bulb. This rendered the whole apparatus inconspicuous. The bulb was operated with the right hand, the pinch cock with the left. A test tube with a sterile cotton plug was always carried, the latter being used to replace the plug which was removed from the intake of the filter at the beginning of the experiment.

The plating was always carried out within 30 minutes after the sample was obtained. The sand from the filter was carefully poured into a 100 cc. flask containing 15 cc. of sterile distilled water. The bolting cloth, which had a tendency to stick to the rubber stopper, was removed with sterile forceps and introduced

into the flask. The contents of the flask were thoroughly shaken and aliquot portions, as shown in table IX, were plated with 10 cc. of +1 nutrient agar. In plating, the introduction of much sand was avoided in the following manner: The end of the pipette was held immediately above the bottom of the flask while the liquid was being drawn up to a point slightly above the graduation mark. After a few seconds, enough of the sandy liquid was allowed to run back into the flask to leave the water just at the mark. During this short interim a large proportion of the sand settled in the tip of the pipette and was returned to the flask as the liquid was lowered to the mark. Blanks were plated several times during the course of the experiments, but no growth developed in any case.

The plates were in all cases incubated for 4 days at 37°C., after which the number of bacterial colonies present in each was determined. Finally, all, or a representative number, of the colonies were examined for the presence of coccus forms. (See table IX.)

The coccus colonies developing on agar are, as a rule, very small and often grow in the deeper strata of the medium. This renders the transfer difficult especially when two are to be made from the same colony—one for the stained preparation and one for the agar slope to be used as a stock culture. The difficulty was partially obviated by subculturing (from all the colonies in certain selected plates) to agar slopes, and incubating the latter at 37°C. After several days an examination served to eliminate the bacilli and moulds, leaving only the coccus cultures which were later examined for the presence of the salivary forms. In this examination the three differential media described above were used.

SOURCES OF SAMPLES

As the investigation in hand seeks to discover a relation between the presence of a characteristic salivary organism and the pollution of air, it was thought best to collect the samples of air under normal conditions, i.e., conditions which are met with in every-day life.

Public conveyances, on account of their usually crowded condition and frequently inefficient ventilation, suggested themselves

as favorable places for tests. Hence, a local street car was chosen as a source for air samples. The often poorly ventilated but well filled motion picture theatres furnished another supposedly promising sampling place. The third locality chosen as a source for air samples was a local 5 and 10 cent store. It was thought that this would furnish an ideal source of contaminated air because of the large crowds of people who are continually voicing their sentiments and desires. In order to determine whether or not the salivary organism is present in an atmosphere which is not in immediate contact with human beings, and which is open to the ventilation of nature,the fourth sample was taken from the open air.

DISCUSSION OF THE EXPERIMENTS

The experiments in table IX are arranged according to the dates on which the tests were made. But for convenience in this discussion the experiments will be taken up according to the source of the samples.

Experiment 1.—This experiment was carried out primarily to test the apparatus. The air sample was taken in a laboratory on the second floor of an old building. There were usually at least two people present in the room, and practically no ventilation was provided, the doors and windows being constantly closed. The apparatus used differed from that used in the remaining experiments in that two sand filters were used in tandem instead of the usual one. During the 15 minutes of operation, 7,800 cc. of air were drawn through the sand of both filters at the rate of 520 cc. per minute.

The sand of the first filter was introduced into 15 cc., that of the second into 6 cc. of sterile distilled water. Quantities of both solutions were plated with the following results:

TABLE VII

Filter number	Plate number	Quantity plated	Total no. of colonies	Coccus colonies
1	1	1 cc.	2	0
1	2	1 cc.	2	2
1	3	2 cc.	4	2
2	4	5 cc.	0	0

The reactions of the cocci isolated from the air in the first filter showed that there was one salivary coccus form present. The remaining three gave negative reactions on the three differential media. It should be noted that out of the 15 cc. of solution from the first filter, only 4 cc. were plated. Eight organisms were present in the quantity examined, making a total of 30 in the entire solution. One characteristic salivary coccus form developed in the portion examined, making, according to the law of averages, a total of 4 in the entire solution. The total volume of air examined being 7,800 cc., the frequency of occurrence of the salivary coccus is 1 in 1,950. According to table VI, the sanitary quality of the air of the room was "probably unsafe" at the particular time at which the sample was taken.

EXPERIMENTS 3, 5, 6, 8, 10

These experiments were carried out in a local street car. The same car line was chosen for all of the experiments in order to eliminate as many variables as possible, such as construction of car, capacity, rate of locomotion, etc. The car was of the ordinary "pay-as-you-enter" type now in use in St. Louis. It had a seating capacity of about 44 people, and could accommodate approximately 40 more standing indoors. The air space in the car in question was about 2,500 cubic feet, or approximately 30 cubic feet for each passenger when the car was filled to its capacity.

As the samples were taken at a time when the outside temperature would not permit the windows to be open, the question of ventilation was carefully studied. As is usually the case, the transoms were tightly closed, and only when the front and rear doors of the car were open at the same time was there an opportunity for a complete renewal of the air. This never happens when the car is in motion, and there is probably never a complete renewal of air unless a strong wind is blowing, thus causing a draught when the car is at a standstill, with both doors open. This particular car was provided with four vents in the roof which could be opened or closed at will. In several of the experiments some of the vents were open; in others, all were closed.

The degree of pollution of the atmosphere in such a car de-

pends, of course, on the amount of coughing, sneezing, speaking, etc., of its occupants. A car may be very crowded but if no coughing, etc., is going on, there will, theoretically, be no pollution of the atmosphere from saliva. Again, if there is much talking, etc., among those present, the atmosphere may be greatly polluted by the dissemination of particles of saliva from the mouth.

The samples were always taken in the early morning between the hours of six and seven, when the majority of the laboring class are on their way to work. The tendency of the passengers at this time of the day is to be quiet, as the morning paper is of absorbing interest to a majority. The samples of air were taken in the center of the car, the opening of the apparatus being about 4 feet from the floor level. In these experiments the apparatus described above was used. In all cases 10,800 cc. of air were drawn through the sand filter at the rate of 900 cc. per minute. The sand was introduced into 15 cc. of sterile distilled water and plated as shown in table IX.

The experiments carried out in street cars will now be taken up in order and the results discussed. If it can be shown that the characteristic salivary organism is present in the air of these cars in sufficient quantity, and if it can later be proved that this salivary organism is not present in the open air, it follows that the atmosphere in these cars is being polluted by the dissemination of particles of saliva from the mouth.

Experiment 3.—While the air sample was being taken for this experiment, 44 people were seated in the car, but none were standing. Out of the 20 colonies appearing on the plate (see table IX), 9 were of bacilli and 11 of coccus forms. Inoculated into the 3 differential media, 8 of the latter reacted negatively in all three media, 1 negatively on lactose and milk, but positively on saccharose, and 2 gave positive reactions in all three media.

It will be recalled that mention has been made of several organisms, both among the salivary and air cocci, which gave a positive reaction with saccharose, but reacted negatively with lactose and milk. The one above referred to as reacting in this manner is probably one of these unidentified coccus forms which seem to be present in both saliva and air. Out of

the 11 cocci present, therefore, two were of the characteristic salivary type, and as only one-third of the sample was plated, a total of 6 may have been present in the entire volume of air examined, or a frequency of occurrence of 1 in 1,800. According to table VI, the sanitary quality of the air was "probably unsafe."

Experiment 5.—During the sampling process for this experiment, 44 persons were seated and approximately 30 standing. Of the 26 colonies which developed on plate 5 (see table IX), 14 were of bacilli, 2 of streptothrix, and 10 of cocci. When transferred to the three differential media, all of the latter gave negative reactions, indicating that the air in the car at the time of this experiment was "safe."

Experiment 6.—At the time of sampling, 44 persons were seated and 30 were standing. On account of the large number of colonies present, only a representative sector of plate 1—comprising one-twelfth of the total area—was examined (see table IX). On this area 21 colonies were counted, 5 bacillus and 16 coccus. On the three differential media, 4 of the latter gave negative reactions throughout, 6 were negative on lactose and milk but positive on saccharose, and 6 gave positive reactions on all three media. It follows that 6 salivary cocci were isolated from one-twelfth of the plate, making a total of 72 from the entire plate, or of 1,080 from the total volume of sand solution,—a frequency of occurrence of 1 in 10. According to table VI, the air in the car at the time of the experiment was "unsafe."

Experiment 8.—The number of persons seated and standing was the same as in experiment 6. On the plate examined (see table IX), 34 colonies developed—15 bacillus and 19 coccus. On the three differential media the coccus forms reacted as follows: Twelve gave negative reactions throughout, 6 were negative on lactose and milk but positive on saccharose, and 1 was negative on lactose and saccharose but positive on milk. The last form was found, after again staining with gentian violet and examining under the microscope, to be a short bacillus. It is to be noted that 6 organisms of the unidentified coccus type were again present. No characteristic salivary cocci were present, thereby marking the air of this particular car as "safe" at the time of the experiment.

Experiment 10.—During this experiment, 44 persons were seated and 15 standing. It was noted that one transom was open. The plate examined (see table IX) gave a total of 12 colonies, of which 5 were of bacilli and 7 of cocci. Of the latter, 6 reacted negatively on all three of the differential media, whereas 1 gave a positive reaction throughout. This makes the frequency of occurrence of the characteristic salivary coccus form 1 in 3,600, and, according to table VI, marks the air in this car as "questionable" at the time of the experiment.

Summarizing the car experiments, it is to be noted that in three out of five cases the characteristic salivary coccus form was isolated, and in such quantity as to mark the air of one "unsafe," that of another "probably unsafe," and of a third "questionable."

EXPERIMENTS 9 AND 11

These experiments were carried out in a local vaudeville house. The construction of the building appeared modern in every respect. The lower floor had a seating capacity of about 2,000, while the balcony accommodated approximately 1,000 people. The house was filled with spectators on the occasions when the samples were taken. Upon inquiry, after the surprisingly good results given below were obtained, it was found that the building was well ventilated by one of the modern appliances for this purpose, whereby the volume of air in the building (about 90,000 cubic feet) was being renewed to a greater or less extent every seven-tenths of a minute. For the collection of the air samples, the same apparatus was used as in the street car experiments, 10,800 cc. of air being drawn through the sand filter at the rate of 900 cc. per minute. The sand was introduced into 15 cc. of sterile distilled water and platings were made as indicated in table IX.

Experiment 9.—The air sample was obtained near the center of the lower floor of the building about 60 feet from the stage. The entire lower floor was packed, and in addition about 100 or more persons were standing in the rear. On the plate examined, a total of 14 colonies developed,—3 mold, 9 bacillus and 2 coccus. Molds were very abundant on the other plates. One of the coccus forms reacted negatively on lactose and milk but positively on saccharose, whereas the other gave negative

reactions on all three differential media. The presence of the single unidentified coccus is again noted. No salivary coccus forms were isolated, from which fact it appears that the air in the particular location from which the sample was taken was "safe."

Experiment 11.—This sample was taken on the balcony of the building, about 10 feet from the rear wall. Every seat was occupied. As indicated in table IX, two plates were examined. On the first, 7 colonies developed—3 streptothrix, 3 bacillus, and 1 coccus. On the second plate 3 colonies appeared, all of which were of bacilli. The reaction of the coccus was negative on the three differential media, thereby indicating that the sample of air taken was free from salivary coccus forms and therefore "safe."

In summing up the results of the experiments carried out in the vaudeville house it is to be noted that in both cases no salivary coccus forms were found. Table IX further shows that the total number of organisms found per unit volume of air was smaller than in the street car experiments.

EXPERIMENTS 4 AND 7

These samples were obtained in the basement of a local 5 and 10 cent store. The ceiling was rather low, being only about 9 feet from the floor level, the entire basement having a volume of about 72,000 cubic feet. The samples in these experiments were taken in the midst of a crowd gathered to listen to a singer advertising songs. Little attention was given to the matter of ventilation until after the results of the experiments were obtained. Subsequently, however, investigation revealed the fact that ample provision had been made for ventilation. Transoms at the level of the sidewalk provide openings to the outside; along the inside wall and near the ceiling are revolving fans about 20 feet apart. These keep the air in circulation until it is drawn out by a suction fan situated in one corner, about 2 feet from the ceiling. The same sampling apparatus was used as in the preceding experiments. As before, a total of 10,800 cc. of air was drawn through the sand filter in each sampling at the rate of 900 cc. per minute. The samples were plated as shown in table IX.

Experiment 4.—The air sample for this experiment was taken

in the midst of a crowd of about 100 people in front of a counter. On the plate examined, a total of 36 colonies developed—16 bacillus and 20 coccus. Of the latter, 18 gave negative reactions throughout on the three differential media, and 2 reacted negatively on saccharose and milk but positively on lactose, the latter sugar being fermented. No salivary coccus forms were isolated, indicating that the air in the basement at the time of the experiment was "safe."

Experiment 7.—This air sample was taken under practically the same conditions as in the previous experiment except that only about 50 people were in the crowd. The plate examined contained 2 streptothrix, 13 bacillus, and 8 coccus colonies. All of the cocci gave negative reactions throughout on the three differential media. No salivary coccus forms were found, which fact leads again to the conclusion that the sanitary quality of the air during the experiment was "safe."

EXPERIMENTS 2, 12, 13, 14

These experiments were performed outdoors. The air sample for experiment 2 was collected in a railroad switch yard at a time when there was no traffic. The samples for experiments 12, 13, and 14 were collected in the immediate vicinity of large storage basins belonging to the local water works and located 300 or 400 feet from the bank of the Mississippi River. The apparatus used was the same as that employed in the previous experiments.

Experiment 2.—The outdoor temperature was 29°F., and while the air sample was being taken it was snowing. A total of 22,500 cc. of air was drawn through the sand filter at the rate of 750 cc. per minute—the operation extending over a period of 30 minutes. Samples were plated as shown in table IX. Of the 3 plates examined, plate 1 yielded 2 bacillus colonies; plate 2, 1 streptothrix, 1 bacillus, and 6 coccus colonies; and plate 4, 2 mold, 1 streptothrix, 1 bacillus, and 2 coccus colonies. All of the coccus forms were grown on the three differential media, 7 giving negative reactions throughout, while 1 reacted positively on saccharose and negatively on lactose and milk. The latter organism is one of the unidentified coccus forms previously referred to. No characteristic salivary cocci were found, in-

dicating that the sanitary quality of the air examined was "safe."

Experiment 12.—At the time the air sample was being taken, a slight drizzling rain was falling, accompanied by considerable wind and a temperature of 45°F. Prior to that time it had been raining continuously for about 24 hours. A total of 10,800 cc. of air was drawn through the sand filter at the rate of 900 cc. per minute, the apparatus meanwhile being held about 5 feet above the ground level. The sand of the filter was introduced into 15 cc. of sterile distilled water, from which platings were made. Table VIII gives the details of the experiment, together with the results obtained.

TABLE VIII

Plate number	Quantity plated	Total no. of colonies	No. of bacteria and molds	Coccus colonies	No. of salivary cocci
1	1 cc.	9	2	7	7
2	1 cc.	3	3	0	0
3	5 cc.	0	0	0	0
4	5 cc.	6	4	2	0

Attention should be called to the fact that on plate 1, in which only 1 cc. of the solution was used, 9 colonies developed—7 coccus and 2 bacillus—, while on plate 4, in which 5 cc. of the solution were used, only 6 colonies appeared,—2 coccus and 4 bacillus. Furthermore, the 7 colonies in plate 1 proved to be of salivary cocci, whereas none of these organisms were present among the cocci of plate 5. These results unquestionably indicatelocal contamination. It is difficult to say just where the contamination took place. Obviously it did not occur during the collection of the sample or even during the mixing of the sand solution; for had this been the case all of the plates should have shown salivary cocci, and the greater number should have occurred on those plates in which larger quantities of the solution were plated. In all probability plate 1 was locally contaminated.

Experiment 13.—While the sample of air was being taken for this experiment, the temperature was 63°F., a light breeze was blowing, and the sky was very cloudy although no rain had

fallen during the preceding 18 hours. A total of 10,800 cc. of air was drawn through the apparatus at the rate of 830 cc. per minute. Samples of the sand solution were plated as shown in table IX. It is to be noted that in those plates containing 1 cc. of the solution no colonies developed, whereas in those containing 5 cc., 1 bacterial colony appeared in each. Attention is called to the consistent results in this experiment to emphasize the fact that the inconsistencies in experiment 12 are due to local contamination. No salivary cocci were found.

Experiment 14.—The air sample for this experiment was taken on a bright, clear day, with a rather strong wind blowing and a temperature of 55°F. A total of 10,800 cc. of air was drawn through the sand filter at the rate of 1,080 cc. per minute. Samples of the sand solution were plated as shown in table IX. Of the 3 coccus forms, 2 gave negative reactions on all three differential media, whereas 1 was positive on saccharose and negative on lactose and milk. The latter will be recognized as one of the unidentified coccus forms. No salivary cocci were isolated.

Summarizing the open air experiments, it is to be noted that, barring the locally contaminated plate 1 in experiment 12, the characteristic salivary coccus form was not isolated; furthermore, that the total number of organisms in the open air is comparatively low.

Summary and Conclusions

Examining the entire series of experiments it appears that in the majority of cases where ventilation was obviously inadequate, the characterisitic salivary coccus form was isolated. On the other hand, the form could in no case be found where ample artificial or natural ventilation existed.

It has been shown that the most characteristic salivary organism can be differentiated and identified; also, that this characteristic organism can be isolated from the air.

In the experiment carried on in one of the street cars in which there were many passengers, the characteristic salivary coccus form was found to be present in such quantities as to indicate that the air in this car was "unsafe." It was later shown that

TABLE IX

DATA ON THE COLLECTION AND EXAMINATION OF AIR SAMPLES

No. of experiment		1	2	3	4	5	6	7
Date of collection		2/12/13	2/22/13	3/15/13	3/15/13	3/19/13	3/22/13	3/22/13
Sampling place		Lab.	Outdoors	Street car	5 and 10c. store	Street car	Street car	5 and 10c store
Temperature (°F.)	Outdoors	60	29	32	36	50	23	50
	Sampling pl.	80	29	45	70	48	45	70
Weather conditions		Sunshine	Snowing	Windy snowing	Windy snowing	Sunshine	Sunshine	Sunshine
Approx. no. of persons	Sitting	0	0	44	1	44	44	1
	Standing	2	0	0	100	30	30	50
Approx. volume of sampling pl. (cu. ft.)		3000		2500	72000	2500	2500	72000
Volume of air exam. (cc.)		7800	22500	10800	10800	10800	10800	10800
Rate of filtration (cc. per min.)		520	750	900	900	900	900	900
No. of organisms in following quantities of sand solution plated	Pl. I. (1 cc.)	2	2	4	30	9	250	4
	Pl. II. (1 cc.)	2	8	3	36	6	250	3
	Pl. III. (2 cc.)	4	0					
	Pl. IV (5 cc.)		6	12	230	25	Too numerous to count.	Spreader
	Pl. V. (5 cc.)			20	200	26	Too numerous to count.	23
Plates examined		I., II. and III.	I., II. and IV.	V.	II.	V.	1/12 of I.	V.
Total col. on plates exam.		8	16	20	36	26	21 on 1/12 of I.	23
No. of bacilli, molds, etc.		4	8	9	16	16	5 on 1/12 of I.	15
No. of cocci		4	8	11	20	10	16 on 1/12 of I.	8
No. of salivary cocci		1	0	2	0	0	6 on 1/12 of I.	0
Frequency of occurrence		1 in 1950	0	1 in 1800	0	0	1 in 10	0
Sanitary quality		Probably unsafe	Safe	Probably unsafe	Safe	Safe	Unsafe	Safe
No. of org. in total vol. of air		30	42	50	570	95	3750	58

TABLE IX (*Continued*)

DATA ON THE COLLECTION AND EXAMINATION OF AIR SAMPLES

No. of experiment		8	9	10	11	12	13	14
Date of collection		3/29/ 3	3/29/13	4/1/13	4/1/13	4/8/13	4/9/13	4/10/13
Sampling place		Street car	Picture show	Street car	Picture show	Outdoors	Outdoors	Outdoors
Temperature (°F.)	Outdoors	41	59	50	77	45	63	55
	Sampling pl.	55	70	63	82	45	63	55
Weather conditions		Sunshine	Cloudy	Sunshine	Sunshine	Rain, very windy	Cloudy	Sunshine, very windy
Approx. no. of persons	Sitting	44	3000	44	3000	0	0	0
	Standing	30	100	15	300	0	0	0
Approx. volume of sampling pl. (cu. ft.)		2530	90000	2500	90000			
Volume of air exam. (cc.)		10800	10800	10800	10800	10800	10800	10800
Rate of filtration (cc. per min.)		900	900	900	900	900	830	1080
No. of organisms in following quantities of sand solution plated	Pl. I. (1 cc.)	34	3	4	7	9	0	2
	Pl. II. (1 cc.)	18	2	2	3	3	0	2
	Pl. III. (2 cc.)	24						
	Pl. IV. (5 cc.)	Spreading mold	14	Spreader	Spreader	0	1	3
	Pl. V. (5 cc.)		8	12	35*	6	1	1
Plates examined		I.	IV.	V.	I. and II.	I., II. and V.	IV. and V.	I., II., IV., and V.
Total col. on plates exam.		34	14	12	10	18	2	8
No. of bacilli, molds, etc.		15	12	5	9	9	2	5
No. of cocci		19	2	7	1	9	0	3
No. of salivary cocci		0	0	1	0	7	0	0
Frequency of occurrence		0	0	1 in 3600	0	1 in 1235	0	0
Sanitary quality		Safe	Safe	Question-able	Safe	†	Safe	Safe
No. of org. in total vol. of air		320	35	42	85	50	2	18

* Abundance of molds.
† Local contamination.

the salivary coccus form could not be found in the open air devoid of the immediate presence of human beings.

It thus appears that the presence of the salivary coccus form in air indicates the presence of man; furthermore, it indicates the pollution of air by particles of mucus from the mouth.

Flügge [1] and his school have shown that pathogenic organisms may be transmitted into the air, and other workers [2] have shown that the tubercle organism is capable of being carried by even such feeble air currents as ordinarily exist in dwellings.

The tubercle organism, as well as the characteristic salivary organism, is present in the saliva of tubercular patients. If, therefore, this salivary organism can be isolated from the air by means of the filter used in the above experiments, does it not follow that the tubercle organism could be isolated in a similar way? Since our manner of breathing is comparable to the operation of the apparatus used, it follows that the tubercle organism may be inhaled by man.

It thus appears that the presence in the air of the most characteristic salivary organism is an index of the possible access of pathogenic organisms to the atmosphere.

In conclusion, the writer wishes to express his thanks to Dr. Geo. T. Moore, for valuable suggestions and numerous courtesies extended during the progress of the work; to Dr. J. R. Schramm, for suggestions, and aid in the preparation of the manuscript; and to Mr. Wilson F. Monfort, Chemist of the City of St. Louis Water Department, for advice given and opportunities provided for the collection and examination of the samples.

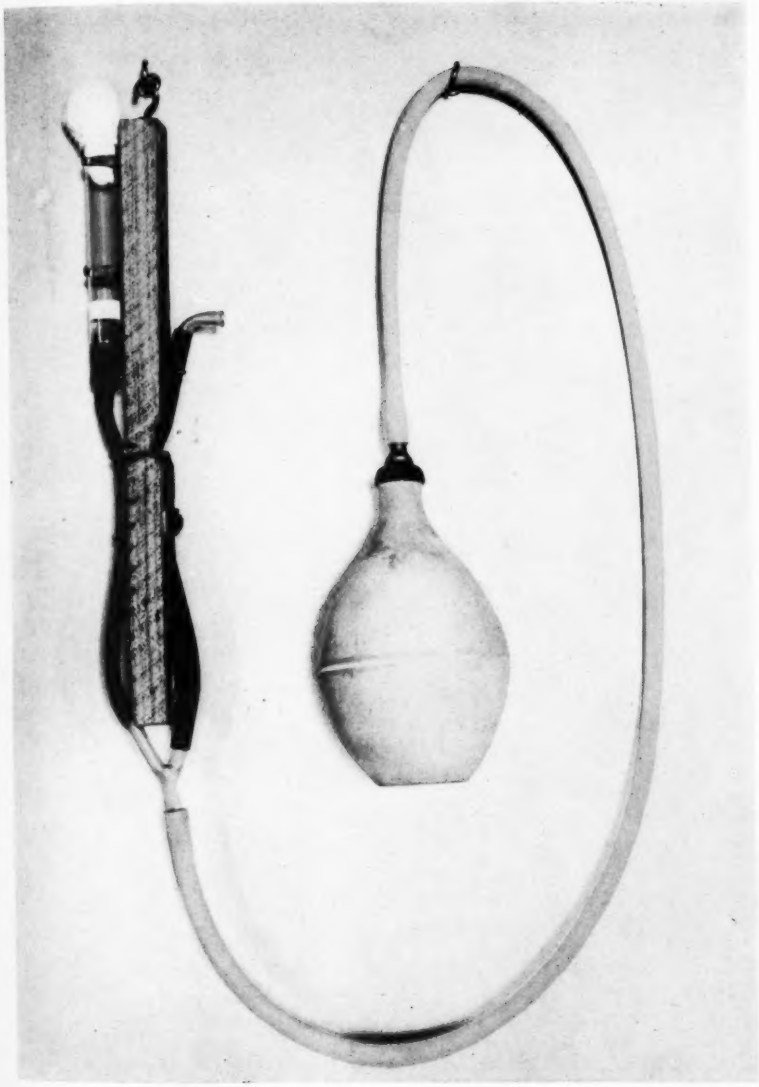
Explanation of Plate

PLATE 2

Air-sampling apparatus showing support, sand filter, pinch cock, exhaust and suction tubes, and pressure bulb.

[1] Gordon, M. H. *loc. cit.*

[2] Kolle and Wassermann, Handbuch der pathogenen Mikroorganismen 1: 169.



NOLTE—SALIVARY ORGANISMS AND AIR POLLUTION

THE POLYPORACEÆ OF OHIO

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INTRODUCTION

The *Polyporaceæ*, or "pore fungi," constitute a relatively small family of the *Basidiomycetes*, characterized by having the spores borne on the interior surfaces of tubes or pores which make up the hymenium of the fungus. In its most comprehensive sense the family embraces the two subfamilies *Boleteæ* and *Polyporeæ*, including also such aberrant genera as *Merulius*, *Porothelium*, *Solenia*, etc. More often the *Boleteæ* are made a separate family, the *Boletaceæ*, usually distinguished from the true *Polyporaceæ* by the more fleshy nature of the plant and by the fact that the pores rather easily separate in a smooth layer from the flesh of the pileus. The true *Polyporaceæ*, on the other hand are more commonly leathery, corky, or woody in texture, and only in rare cases are the tubes separable from the context. More recently Dr. Murrill, who has monographed the North American species of the family for the North American Flora—now being issued by the New York Botanical Garden—, has still further limited the family so as to exclude not only the genera referred to above, but also certain of the true polypores which possess a more or less gelatinous or waxy hymenium. For the reception of certain of these forms he has erected the family *Xylophagaceæ*.

C. G. Lloyd has published monographic papers on certain of the sections of the family, using for the most part as the generic names, the sectional names given by Fries. Within the past year a third system of classification has been proposed by Miss Ames, of Cornell University, who divides the family into groups on the character of the context, and these groups are separated into genera on the form of the fruit body, surface modifications, spore characters, etc. Various workers in Europe have at-

tempted to revise the genera of the *Polyporaceæ* but none of these classifications have been generally adopted by mycologists.

The family is here taken to include the following genera: *Polyporus* (including *Polystictus*), *Fomes*, *Trametes*, *Dædalea*, *Lenzites*, *Cyclomyces*, *Favolus*, *Glœoporus*, *Merulius*, and *Irpex*. Distributed among these genera are practically one hundred species found within the state. Of these, 78 have been collected by the writer,4 others have been sent in by correspondents, and examination has been made of collections of 5 other species taken within the state and preserved either at the Lloyd Museum at Cincinnati, or in the herbarium of the New York Botanical Garden. Of the remaining 12 species some are known only from the records left by Morgan, Lea, Montagne, Berkeley, and Kellerman, others are admitted because there is every reason to believe that they will be found within the state since they are known to have been collected in nearby counties of adjoining states.

The resupinate *Polyporaceæ*, usually included in the genus *Poria*, have been omitted from this paper. Very little is known in this country concerning these forms and very few authentic specimens were available for study and comparison. Most of the species that have been reported from this country have been based on scarcely more than a guess, and it is impossible for the amateur mycologist to determine his material from the confused and often fragmentary account that has been written. Until the genus has been thoroughly studied by a competent mycologist, only added confusion would result from anything more than a reference to it in this paper.

In the preparation of the keys, relationships, both of genera and species, have been entirely ignored, the aim being to produce a usable key rather than to exhibit relationships. The writer believes that the color of the context is one of the most constant of the gross characters of these plants, and the genera are divided into sections on that basis. The presence or absence of a stipe, the duration of the plant, the hymenial configuration, the surface markings of the pileus, etc., are brought into the key in an order which the writer believes corresponds to their relative importance as specific distinguishing characters. Spore characters, especially spore colors, are not used in the separation

of the genera, and in the separation of the species only where experience has shown that the spores are always easily obtained. In many cases it is impossible to obtain spores, especially if they be uncolored, from the hymenium of dried plants. However, when plants are taken in the fresh condition it is usually a simple matter to obtain them by leaving the fungus over night in a moist atmosphere and allowing the spores to fall upon a glass slide. Spores of the perennial woody forms may often be obtained by this method when an examination of the same material in the dried state does not reveal their presence. In this paper spore measurements have been freely taken from other publications, both European and American. This was done in order that the descriptions might be made more comparable. Due credit is given to the author in every case where this was done.

An effort has been made to make the descriptions exactly comparable one with another. For this purpose a definite sequence of presentation has been arranged for the different characters and this order preserved in all but a few instances in which entire descriptions were taken from the original sources. In the comments following each species the characteristic specific distinctions are pointed out and references are made to illustrations of one sort or another that give a good idea of the plant as the writer understands it. Practically all of these references are to papers published in this country. The writer has had access to all of the important publications on the family, both European and American. Most of the European writings are not available to a large part of those students for whom this paper is intended and it was believed that a careful selection of citations to the illustrations published in this country would be of more value than citations to the less known and often inaccessible European publications. Those who are in a position to look up additional references will have access as well to volumes 19 and 20 of Saccardo's 'Sylloge Fungorum,' where an exhaustive index to illustrations will be found.

It is believed that there can be no question of the need of a paper worked out along the above indicated lines. No such publication exists for any state in the Union and the only aids that students have had in determining their collections have

been either the incomplete "mushroom" books or such extensive works as 'Sylloge Fungorum' and in more recent years the monograph presented in the 'North American Flora.'

In the matter of citation and nomenclature an attempt has been made to follow the rules and recommendations of the International Botanical Congress at Brussels. Since there has been little opportunity to compare specimens of our plants with those of Europe or with type specimens, the procedure in the matter of synonymy has been very conservative. The only names cited as synonyms are those of which the writer has a personal knowledge gained from the examination of authentic material, usually species described from Ohio. Where there has been a doubt as to the identity of a plant in this country with that of one in the old world the procedure has been to use the name under which it has been described or known in this country.

The first and therefore the most complete set of specimens is in the herbarium of the writer; a set of all of the more common forms is in the herbarium of Dr. Bruce Fink, of Miami University, at Oxford, Ohio; a partial set is in the state herbarium, at Columbus; and a large number of species, sent to Dr. Murrill for determination and verification, are in the herbarium of the New York Botanical Garden.

The writer is under deep obligations to the following persons in various ways: First of all to Dr. Bruce Fink, under whose direction the work was begun, whose aid, criticism, and advice has made this publication possible; to Dr. W. A. Murrill, of the New York Botanical Garden, for many kindnesses in verifying and determining specimens sent to him, and for the privilege of studying the specimens in the herbarium at that place; to Mr. C. G. Lloyd, of Cincinnati, for the privilege of working in the Lloyd Library and Museum and for determinations of specimens; to Rev. G. Bresadola, of Trient, Tyrol, for determination of specimens; to Dr. E. A. Burt, of the Missouri Botanical Garden, for access to his herbarium and for suggestions as to the final form of the paper; and to all who have aided in the work by sending specimens and in various other ways.

It is hoped that the paper will be found useful not only to Ohio students but in the neighboring states of the Great Lakes

region and in the Ohio valley as well. It is with this idea in mind that the paper has been prepared.

Key to the Genera.

Sporophore entirely resupinate; pileus none ..... *Poria*[1]
Sporophore sessile or stipitate, sometimes effused-reflexed but not normally entirely resupinate 1
1. Hymenium composed of concentric lamellæ; pileus stipitate .. *Cyclomyces* p. 147
1. Hymenium not composed of concentric lamellæ; pileus sessile or stipitate ... 2
2. Hymenium not distinctly poroid, the pores reduced to shallow pits separated by narrow ridges or reticulations ..... *Merulius* p. 150
2. Hymenium distinctly poroid, irpiciform, dædaloid or lamellate, but not pitted 3
3. Hymenium more or less waxy or gelatinous, the layers of tubes separating smoothly from the context in fresh specimens or when moistened; pileus sessile, thin and flexible ..... *Glœoporus* p. 149
3. Hymenium not at all waxy or gelatinous and not separating smoothly from the context; pileus sessile or stipitate 4
4. Hymenium either dædaloid, labyrinthiform or lamellate, at least in part 5
4. Hymenium poroid or sometimes broken up into teeth 9
5. Context white 6
5. Context brown 8
6. Pileus minutely velvety to glabrous; context more than 1 mm. thick *Dædalea* p. 143
6. Pileus hirsute to villous; context 1 mm. or less thick 7
7. Hymenium lamellate, at least in part ..... *Lenzites* p. 145
7. Hymenium dædaloid but never lamellate ..... *Dædalea* p. 143
8. Plants woody and perennial, more than 1 cm. thick; hymenium not at all lamellate ..... *Trametes* p. 138
8. Plants coriaceous or corky, less than 1 cm. thick; hymenium often lamellate ..... *Lenzites* p. 145
9. Hymenium broken up into teeth 10
9. Hymenium poroid, not broken up into teeth 13
10. Tubes or teeth 5 mm. or more long ..... *Irpex* p. 151
10. Tubes or teeth less than 5 mm. long 11
11. Hymenium labyrinthiform at first and remaining so at the margin *Dædalea* p. 143
11. Hymenium never labyrinthiform 12
12. Pileus less than 1 cm. broad; fungus mostly resupinate ..... *Irpex* p. 151
12. Pileus more than 1 cm. broad; fungus not mostly resupinate *Polyporus* p. 86
13. Pores large and hexagonal; stipe present 14
13. Pores small and circular or angular; stipe present or absent 15
14. Stipe lateral, often rudimentary; pores usually radiating and longer in the radial direction ..... *Favolus* p. 148
14. Stipe usually central or subcentral; pores not radiating .... *Polyporus* p. 86
15. Tubes in a single layer; plants annual 16
15. Tubes in two to several layers; plants perennial 17

[1] See Introduction p. 82.

16. Tubes not in a distinct stratum but appearing to be sunken to different depths into the context ..... *Trametes* p. 138
16. Tubes forming a well marked stratum entirely distinct from the context ..... *Polyporus* p. 86
17. Hymenium bright yellowish brown; plants growing only on the wood of coniferous trees ..... *Trametes* p. 138
17. Hymenium whitish, flesh-colored, dull brown, etc., but not bright yellowish brown; plants growing on the wood of either coniferous or deciduous trees ..... *Fomes* p. 126

DESCRIPTIONS AND KEYS TO THE SPECIES

POLYPORUS Mich. ex Fries,
Syst. Myc. 1: 341. 1821; Mich. Nov. Plant. Gen. 129. 1729.

Plants annual or in rare cases persisting for two or three years, terrestrial or epixylous, sessile or stipitate; pileus fleshy, coriaceous or corky in texture, small or of immense size, often brightly colored; context white, yellow, red, or brown; tubes in a single layer, all sunken into the context to an equal depth so that their bases form a definite continuous straight line; mouths mostly circular or angular, in rare cases showing a favoloid or daedaloid tendency and sometimes breaking up into teeth; stipe (when present) variable in position and texture; spores white (bluish in one species), or some shade of brown.

KEY TO THE SPECIES

Context white or whitish ..... Section I.
Context reddish or yellowish ..... Section II.
Context brown or brownish ..... Section III.

Section I.

Sporophore stipitate or substipitate 1
Sporophore sessile or sometimes effused-reflexed but never stipitate 21
1. Pileus and stipe covered with a reddish varnish 2
1. Pileus and stipe not red-varnished 3
2. Varnish disappearing with age, the pileus then whitish or yellowish *61. P. Curtisii*
2. Varnish persisting, the pileus not changing color ..... *60. P. lucidus*
3. Plant small, not more than 1 cm. high ..... *29. P. pocula*
3. Plant always much larger 4
4. Stipe compound, branching near the base; pileoli usually several or many. 5
4. Stipe simple or not branching more than once; pileus generally single 9
5. Pileoli small (usually less than 5 cm. broad) and numerous 6
5. Pileoli large (5 cm. or more broad) and few in number 7

6. Pileoli regular in outline and centrally attached; the branches of the stipe regular and cylindrical in form ..... *38. P. umbellatus*
6. Pileoli always laterally attached; the stipe branches irregular. *39. P. frondosus*
7. Spores roughly echinulate ..... *41. P. Berkeleyi*
7. Spores smooth 8
8. Pileus pallid or light brown; hymenium usually turning black where bruised and on drying ..... *40. P. giganteus*
8. Pileus yellowish green; hymenium not turning black ..... *37. P. flavovirens*
9. Context soft and spongy above, firm next to the hymenium; plants often much distorted; usually growing about stumps ..... *28. P. distortus*
9. Context uniform; plants not distorted 10
10. Plants growing on the ground 11
10. Plants growing on wood 12
11. Stipe black and rooting at the base; pileus some shade of brown .. *36. P. radicatus*
11. Stipe not black and rooting at the base; pileus yellowish green .. *37. P. flavovirens*
12. Sporophore more or less globose; tubes concealed by a volva .. *27. P. volvatus*
12. Sporophore not globose; volva absent 13
13. Sporophore arising from a cup-shaped, sterile body that sometimes disappears; pileus white; found only on dead branches of *Ulmus* ..... *6. P. conchifer*
13. Sporophore not arising from a cup-shaped sterile body 14
14. Margin of the pileus projecting 5 mm. or more beyond the hymenium; hymenium separating smoothly from the context in fresh specimens; growing only on *Betula* ..... *26. P. betulinus*
14. Plants not as above 15
15. Hymenium bright sulphur-yellow ..... *42. P. sulphureus*
15. Hymenium not bright sulphur-yellow 16
16. Mouths of the tubes minute, averaging 4-7 to a mm. 17
16. Mouths of the tubes larger, averaging 1-3 to a mm. 18
17. Mouths of the tubes averaging 4 to a mm.; pileus rarely more than 5 cm. in diameter ..... *35. P. elegans*
17. Mouths of the tubes averaging about 6 to a mm.; pileus 4–20 cm. in diameter ..... *34. P. picipes*
18. Pileus large, more than 5 mm. thick; plant growing on living trees; stipe black at the base ..... *33. P. squamosus*
18. Pileus small or medium sized, not more than 5 mm. thick; stipe not black at the base 19
19. Tubes long-decurrent on the stipe; context soft and friable when dry ..... *32. P. pennsylvanicus*
19. Tubes slightly or not at all decurrent; context not soft and friable when dry .. 20
20. Pileus yellowish brown; mouths of the tubes almost 1 mm. in diameter; walls thin ..... *31. P. arcularius*
20. Pileus darker than above, sometimes sooty-black; mouths of the tubes averaging 2 to a mm.; walls at first thick ..... *30. P. brumalis*
21. Pileus red-varnished, at least when young 22
21. Pileus never red-varnished 23
22. Varnish disappearing with age, the pileus then whitish or yellowish ..... *61. P. Curtisii*
22. Varnish persistent, the pileus not changing color ..... *60. P. lucidus*
23. Sporophore more or less globose; tubes concealed by a volva ..... *27. P. volvatus*
23. Sporophore not globose; volva absent 24

24. Sporophore arising from the under side of a cup-shaped, sterile body; found only on dead branches of *Ulmus*.....*6. P. conchifer*
24. Sporophore not arising from a cup-shaped, sterile body..... 25
25. Margin of the pileus projecting 5 mm. or more beyond the hymenium; hymenium separating smoothly from the context in fresh specimens; found only on *Betula*.....*26. P. betulinus*
25. Plants not as above..... 26
26. Hymenium bright sulphur-yellow.....*42. P. sulphureus*
26. Hymenium not bright sulphur-yellow..... 27
27. Pileus distinctly brown in color; context usually light brown; hymenium changing color when bruised.....*46. P. resinosus*
27. Pileus not brown in color; hymenium never changing color when bruised .. 28
28. Hymenium more or less smoke-colored or black..... 29
28. Hymenium not at all smoke-colored or black..... 32
29. Pileus more than 4 mm. thick..... 30
29. Pileus not more than 4 mm. thick..... 31
30. Context fragrant, with the odor of anise.....*23. P. fragrans*
30. Context not fragrant, odor sometimes disagreeable.....*24. P. fumosus*
31. Mouths of the tubes angular, minute, averaging 5–7 to a mm.; dissepiments thin.....*22. P. adustus*
31. Mouths of the tubes circular or subcircular, medium sized, averaging 3–5 to a mm.; dissepiments thick.....*24. P. fumosus*
32. Context fibrous or coriaceous in fresh plants; pileus never more than 1.5 cm. thick, and usually much thinner..... 33
32. Context either soft, spongy and full of water or firm and corky, often fragile when dry; pileus often more than 1.5 cm. thick..... 43
33. Hymenium broken up into teeth..... 34
33. Hymenium entire or lacerate but not broken up into teeth..... 36
34. Context more than 1 mm. thick.....*9. P. biformis*
34. Context not more than 1 mm. thick.....35
35. Plants growing only on the wood of coniferous trees.....*2. P. abietinus*
35. Plants growing only on the wood of deciduous trees.....*3. P. pargamenus*
36. Context 1 mm. or less thick..... 37
36. Context more than 1 mm. thick..... 40
37. Mouths of the tubes minute, averaging 4–6 to a mm.; hymenium never violet or purple..... 38
37. Mouths of the tubes larger, averaging 2–3 to a mm.; hymenium often violet or purple..... 39
38. Surface of the pileus villous or velvety; pileus multizonate, generally more than 2 cm. broad.....*1. P. versicolor*
38. Surface of the pileus densely hirsute; pileus azonate or with one or two zones, generally less then 2 cm. broad.....*4. P. hirsutulus*
39. Plants growing only on the wood of coniferous trees.....*2. P. abietinus*
39. Plants growing only on the wood of deciduous trees.....*3. P. pargamenus*
40. Mouths of the tubes large, averaging 1–2 to a mm.....*9. P. biformis*
40. Mouths of the tubes medium sized, averaging 3–4 to a mm..... 41
41. Tubes more than 2 mm. long.....*7. P. pubescens*
41. Tubes not more than 2 mm. long..... 42
42. Surface of the pileus velvety to hirsute.....*5. P. hirsutus*
42. Surface of the pileus minutely pubescent or glabrous.....*8. P. Lloydii*

43. Plants mostly resupinate 44
43. Plants not mostly resupinate 45
44. Pileus azonate, margin often inrolled ..... *10. P. semipileatus*
44. Pileus zonate, margin always straight ..... *21. P. zonalis*
45. Pileus corky in texture when fresh, usually rather thick and firm 46
45. Pileus soft and spongy in texture when fresh 49
46. Pileus distinctly encrusted; hymenium and context pinkish or rosy when fresh; plants usually growing on *Fraxinus* ..... *P. fraxineus*[1]
46. Pileus not encrusted; hymenium and context whitish when fresh; plants not usually on *Fraxinus* 47
47. Pileus more than 2 cm. thick; tubes more than 4 mm. long ..... *25. P. robiniophila*
47. Pileus not more than 2 cm. thick; tubes not more than 4 mm. long 48
48. Plants with a sweet anise odor ..... *23. P. fragrans*
48. Plants with no odor, or odor disagreeable ..... *24. P. fumosus*
49. Mouths of the tubes minute, averaging 6–7 to a mm.; plants with a sweet acid odor ..... *14. P. galactinus*
49. Mouths of the tubes larger, averaging 1–4 to a mm. 50
50. Pileus generally less than 4 cm. broad 51
50. Pileus generally more than 4 cm. broad 53
51. Pileus pubescent; mouths of the tubes dentate, lacerate, or irregular 52
51. Pileus glabrous; mouths of the tubes entire; plants with a sweet acid odor *12. P. chioneus*
52. Pileus and spores (in mass) often bluish or slate-colored; tubes equalling in length the thickness of the context ..... *11. P. caesius*
52. Pileus and spores pure white; tubes shorter in length than the thickness of the context ..... *13. P. lacteus*
53. Plants growing only on the wood of coniferous trees 54
53. Plants growing only on the wood of deciduous trees 55
54. Tubes usually more than 5 mm. long, the mouths averaging 2–3 to a mm. *19. P. borealis*
54. Tubes usually less than 5 mm. long, the mouths averaging 4–5 to mm. *18. P. guttulatus*
55. Margin of the pileus thick and rounded ..... *17. P. obtusus*
55. Margin of the pileus thin and acute 56
56. Mouths of the tubes large, averaging 1–2 to a mm. ..... *16. P. delectans*
56. Mouths of the tubes small, averaging 3–5 to a mm. 57
57. Fresh plant with a disagreeable odor; context very hard when dry *20. P. Spraguei*
57. Fresh plant with no disagreeable odor ..... *15. P. spumeus*

Section II

Pileus and hymenium deep cinnabar-red 1
Pileus and hymenium not deep cinnabar-red (rosy or orange-colored in some species) 2
1. Pileus less than 5 mm. thick, often zonate ..... *44. P. sanguineus*
1. Pileus more than 5 mm. thick, never zonate ..... *45. P. cinnabarinus*
2. Hymenium bright sulphur-yellow in fresh plants ..... *42. P. sulphureus*
2. Hymenium not bright sulphur-yellow 3
3. Plant growing only on the wood of *Quercus* and *Castanea*; pileus yellowish or orange-colored ..... *43. P. Pilotæ*

[1] For description see p. 130 under the genus *Pomes*.

3. Plant growing usually on *Fraxinus;* pileus usually stained more or less with red ..... *P. frazineus*[3]
3. Plant growing usually on coniferous wood; rose-colored without and within... *P. carneus*[3]

Section III

Pileus stipitate or substipitate 1
Pileus sessile or effused-reflexed, not stipitate 9
1. Pileus and stipe covered with a reddish varnish at least when young 2
1. Pileus and stipe not red-varnished 3
2. Pileus and stipe at first red-varnished, the varnish disappearing and the pileus becoming whitish or yellowish when mature ..... *61. P. Curtisii*
2. Pileus and stipe strongly red-varnished, the varnish not disappearing with age ..... *60. P. lucidus*
3. Context not more than 1 mm. thick; plants growing on the ground 4
3. Context more than 1 mm. thick; plants growing on wood or attached to buried wood 6
4. Surface of the pileus marked with silky striations ..... *59. P. cinnamomeus*
4. Surface of the pileus not silky 5
5. Mouths of the tubes small, averaging 2–4 to a mm.; tubes usually less than 3 mm. long ..... *58. P. perennis*
5. Mouths of the tubes large, averaging 0.5–1 mm. or more in diameter; tubes usually more than 3 mm. long ..... *57. P. focicola*
6. Surface of the pileus distinctly encrusted ..... *P. lobatus*[4]
6. Surface of the pileus not at all encrusted 7
7. Context decidedly duplex, spongy above, firm next to the tubes... *55. P. circinatus*
7. Context not duplex 8
8. Hymenium some shade of yellow (yellowish brown, yellowish green, etc.), quickly changing color when bruised; growing on or about trees and stumps of *Pinus;* spores white ..... *54. P. Schweinitzii*
8. Hymenium cinereous to brownish, not changing color when bruised; growing on the ground or attached to buried wood; spores brown ..... *56. P. obesus*
9. Pilei forming a densely imbricate, globose or cylindrical mass ..... *P. graveolens*[5]
9. Pilei not forming a densely imbricate, globose or cylindrical mass 10
10. Pileus red-varnished ..... *60. P. lucidus*
10. Pileus not red-varnished 11
11. Pileus distinctly encrusted ..... *P. lobatus*[4]
11. Pileus not distinctly encrusted 12
12. Plants growing on or about stumps or trunks of *Pinus*... *54. P. Schweinitzii*
12. Plants growing on wood of deciduous shrubs or trees, often on living trunks 13
13. Context usually less than 7 mm. thick; plants small or medium sized 14
13. Context more than 7 mm. thick; plants large 17
14. Spores white 15
14. Spores brown 16

[1] See p. 130 for a description of this plant.
[2] For description of this plant see p. 131 under the genus *Fomes*.
[3] This plant is described on p. 137 under the genus *Fomes*.
[4] For description see p. 131 under the genus *Fomes*.
[5] For description see p. 137 under the genus *Fomes*.

15. Pileus spongy and watery when fresh; context friable when dry; mouths of the tubes averaging 2–4 to a mm. ..... *47. P. nidulans*
15. Pileus firm and rigid; context corky when dry; mouths of the tubes minute, averaging 5–8 to a mm. ..... *48. P. gilvus*
16. Plants growing on the wood of *Alnus* and *Betula*; spores light brown... *49. P. radiatus*
16. Plants growing on the wood of *Acer*, *Fagus*, and other deciduous trees... *50. P. cuticularis*
17. Context very light brown ..... *46. P. resinosus*
17. Context yellowish brown or darker 18
18. Surface of the pileus hirsute; plants growing on various diseased deciduous trees ..... *51. P. hispidus*
18. Surface of the pileus fibrillose or glabrous; plants growing only on the wood of *Quercus* 19
19. Sporophore medium sized, less than 10 cm. broad and 3 cm. thick..... *53. P. dryophilus*
19. Sporophore large, more than 10 cm. broad and 3 cm. thick..... *52. P. dryadeus*

1. P. versicolor L. ex Fries, Syst. Myc. 1: 368. 1821.
Boletus versicolor L. Sp. Plant. 1176. 1753.

Pileus sessile or effused-reflexed, imbricate or single, dimidiate or encircling twigs and then often orbicular by confluence, 2–5 x 2–7 x 0.1–0.3 cm., coriaceous, prevailing color grayish, but marked by many narrow, multicolored zones, ranging from white to yellow, brown, reddish, greenish, blackish, etc., villous or velvety, the margin thin and acute, usually sterile below; context white or whitish, fibrous, less than 1 mm. thick; tubes 1–2 mm. long, the mouths white or yellowish, sometimes somewhat glistening, circular to angular, averaging 3–5 to a mm., the walls thin, entire or slightly lacerate; spores white, smooth, oblong, sometimes curved, 1.2–2 x 5–6.3 μ.

On all kinds of dead wood. Common throughout the year.

Easily distinguished by the multizonate, multicolored pileus. *P. hirsutulus* Schw. is often considered to be a form of this species. *P. zonatus* Fries, as reported by Morgan, is one of the many forms of it. The following references contain good illustrations of our plant: Hard, Mushrooms *f. 343.*, White, Hymen. Conn. *pl. 36.*, and Moffatt, Higher fungi of the Chicago region *pl. 17. f. 1.*

2. P. abietinus Dicks. ex Fries, Syst. Myc. 1: 370. 1821.
Boletus abietinus Dicks. Fasc. Pl. Crypt. Brit. 3: 21. 1793.

Pileus sessile or effused-reflexed, dimidiate and broadly attached, or flabelliform and attached by the attenuate base

of the pileus, 0.5–5 x 0.5–5 x 0.1–0.2 cm., coriaceous, white to cinereous or almost black behind, villous, zonate, margin thin and acute; context white or pallid, fibrous, not more than 1 mm. thick; tubes less than 3 mm. long, the mouths white to bay and often violaceous toward the margin, averaging 2–3 to a mm., the dissepiments thin and soon lacerate and breaking up into teeth.

Growing only on the wood of coniferous trees. In autumn. Rare.

Closely related to *P. pargamenus* Fries, from which it is most easily separated by the habitat. The following spore dimensions are found in the literature: Karsten—"oblong 4–6 x 1–3 μ"; Murrill—"globose, smooth, hyaline, 4.5–5.5 μ in diameter"; Bresadola—"hyaline, cylindrical, subcurved, 6–7 x 2.5 μ."

3. P. pargamenus Fries, Epicr. Syst. Myc. 480. 1838.

Pileus sessile or effused-reflexed, imbricate, dimidiate or flabelliform, sometimes attached by an attenuate base, 1–7 x 1–7 x 0.1–0.4 cm., coriaceous, whitish to cinereous or yellowish brown, villous, zonate, the zones sometimes differently colored, margin very thin, acute, broadly sterile below, often violaceous in color; context white or whitish, fibrous, very thin, less than 1 mm. thick; tubes not more than 2.5 mm. long, the mouths whitish to bay and often violaceous toward the margin, angular, averaging 2–3 to a mm., the dissepiments thin and soon breaking up into teeth; spores white, smooth, oblong, slightly curved, 2–2.5 x 5–6.3 μ.

Growing on the wood of deciduous trees, especially of *Quercus* and *Prunus*. September to December. Common.

Close to *P. abietinus* Dicks. ex Fries, but usually found on dead wood of deciduous trees. Well represented by Hard (Mushrooms *f. 345*) as *P. pergamenus*.

4. P. hirsutulus Schw. Trans. Am. Phil. Soc. II. 4: 156. 1832.

Pileus sessile or effused-reflexed, often imbricate, dimidiate, 0.5–2 x 0.5–2.7 x 0.1–0.2 cm., coriaceous, gray or cinereous to yellowish brown, hirsute or strigose, azonate or with 2–3 colored zones, margin thin and acute, usually sterile below; context white or whitish, membranous, less than 1 mm. thick; tubes less than 2 mm. long, mouths whitish to yellowish, rarely

glistening, circular or angular, averaging 3–5 to a mm., the dissepiments thin and entire.

On dead branches of deciduous trees, more often on fruit trees. Found from August to December. Not common.

Separated from *P. versicolor* L. ex Fries, by the more hirsute or strigose pubescence on the pileus, and by the smaller size. Specimens collected at Cincinnati by D. L. James and referred to *P. velutinus* Fries are now referred to this species.

5 **P. hirsutus** Wulfen, ex Fries, Syst. Myc. 1: 367. 1821.

Boletus hirsutus Wulfen, in Jacq. Coll. 2: 149. 1788.

Pileus sessile, or effused-reflexed, dimidiate, 1.5–5 x 1.5–7 x 0.2–1 cm., flexible when moist, firm and sometimes rigid when dry, grayish to yellowish or smoky brown, hirsute or tomentose, sometimes zonate, sometimes concentrically sulcate, the margin thin or rather thick, acute, sometimes dark colored; context white or pallid, tough to soft-corky, 1–6 mm. thick; tubes 1–4 mm. long, the mouths white, grayish or fuliginous, circular to somewhat angular, averaging 3–4 to a mm., the walls rather thick and always entire; spores white, smooth, cylindrical, often curved, 2.5 x 5–8 μ.

On dead wood of deciduous trees. Found throughout the year.

From closely related species with a conspicuous hairy covering this plant is perhaps most easily separated by the persistently thick walled tubes that never become torn or lacerate. Any plant with the characteristics of this group and possessing the dark-colored marginal band to which reference is made in the description may always with safety be referred to this species. From *P. versicolor* L. ex Fries, the plant is separated by the absence of the numerous multicolored zones. Hard's figure (Mushrooms *f. 342*) is not a good illustration of our plant. Murrill describes the plant under the name of *Coriolus nigro-marginatus* (Schw.) Murr.

6. **P. conchifer** Schw. ex Fries, Epicr. Syst. Myc. 463. 1838.

Boletus conchifer Schw. Syn. Fung. Car. 98. 1822.

Pileus sessile or attached by a lateral tubercle and then appearing substipitate, reniform to dimidiate in outline, 1–3 x 1–4 x 0.1–0.3 cm., coriaceous, white to yellowish, glabrous,

zonate or azonate, the margin very thin and acute; on the upper surface and at the base of the pileus a small cup-shaped or disk-like sterile structure is usually borne, white or brown and often zoned on the inside; context white, fibrous, less than 1 mm. thick; tubes not more than 2 mm. long, at first white, often yellowish on drying, the mouths angular and thin-walled averaging about 3 to a mm., the dissepiments often lacerate; stipe (?) rudimentary, tubercular; spores not obtained.

Growing only on fallen branches of *Ulmus*. Common.

This plant has somewhat the appearance of *P. pubescens* Schum. ex Fries, from which, however, it is easily separated by the much thinner pileus, the attenuate base, the presence of the sterile cup, and the habitat. The cup is sometimes absent. The development of the cup has not been closely followed. Lloyd believes that the fertile pileus is first developed and from it the sterile cup arises, and that during the winter the fertile portion falls away, the cup persisting on the substratum but not giving rise to new pilei the next season. Miss Ames comes to the conclusion that the sterile cups represent pilei whose marginal hyphæ have been killed by unfavorable conditions and which as a result may develop a fruiting surface from the base of the dead cup-like pileus. This would explain the occasional absence of the sterile cup, its presence depending upon the death of the marginal hyphæ in the early stages of the production of a first pileus. *P. virgineus* Schw. described from North Carolina is said to be this plant. The plant is exceptionally well illustrated by Lloyd (Myc. Notes, Polyporoid Issue 3 *f. 365–66*), and by Moffat (Higher fungi of the Chicago region *pl. 16. f. 2*).

7. P. pubescens Schum. ex Fries, Syst. Myc. **1**: 367. 1821.
Boletus pubescens Schum. Enum. Pl. Saell. **2**: 384. 1803.
Polyporus Sullivantii Mont. Ann. Sci. Nat. II. **18**: 243. 1842.

Pileus sessile, dimidiate, 1.5–5 x 2.5–5 x 0.4–1 cm., fleshy-tough when fresh, firm when dry, white or yellowish in fresh specimens, sometimes umber or brown when dry, villous-tomentose, zonate or azonate, margin thin, acute; context white or pallid, fibrous-tough when fresh, more firm when dry, 1–5 mm. thick; tubes 1–4 mm. long, the mouths white, yellowish, or umber, angular, averaging 3–4 to a mm., the

dissepiments thin, entire to dentate; spores white, smooth, cylindrical, curved, 2.7–3.6 x 5.4 μ.

On dead wood of deciduous trees. August to November. Common.

Plants collected in the Miami valley by Morgan and referred by him to *P. velutinus* Fries belong here. Plants distributed by Kellerman in his 'Fascicles of Ohio Fungi' as *P. molliusculus* Berk. are referred to this species. *P. fibula* Fries as reported by Morgan is probably the same as *P. pubescens* var. *Grayii*, here included under *P. pubescens*. Hard (Mushrooms *f. 339*) gives a good illustration of the plant.

8. P. Lloydii (Murr.) Overholts, n. comb.

Coriolus Lloydii Murrill, N. Am. Flora **9**: 23. 1907.

Pileus rather thin, laterally connate, rigid, tough, cuneate to flabelliform, applanate, tubercular-sessile, 2–3 x 3–4 x 0.2–0.4 cm.; surface white or isabelline, scabrous, somewhat rugose, marked with a few narrow, indistinct, pale latericeous zones; margin thin, fertile, irregular, lobed; context punky-fibrous, white, 1.5–2 mm. thick; tubes 1–1.5 mm. long, white within, mouths angular, subglistening, 4 to a mm., edges thin, firm, dentate, white or isabelline; spores globose, smooth, hyaline, 2 μ; hyphæ 5 μ.

On dead wood. Rare.

The above description is taken from the 'North American Flora.' The type specimens were collected near Cincinnati, Ohio, by C. G. Lloyd, and to the writer's knowledge the plant has not been found since. The species appears to be distinct.

9. P. biformis Klotzsch, Linnaea **8**: 486. 1833.

P. molliusculus Berk. Hooker's Lond. Jour. Bot. **6**: 320. 1847.

Plants sessile, effused-reflexed or resupinate, often imbricate; pileus dimidiate or laterally confluent and elongate, 0–5.5 x 1.5–6 x 0.2–1.5 cm., soft and pliable when fresh, slightly flexible to rigid when dry, white, pallid, bay, or ochraceous, appressed-fibrillose, usually rough, azonate or subzonate, the margin thin and acute; context white or whitish, fibrous-tough when fresh, soft-corky when dry, 1–5 mm. thick; tubes white, becoming bay on drying, 2–5 mm. long, the mouths circular to angular or sinuous, averaging 1–2 to a mm., the dissepiments

rather thin and usually becoming lacerate and broken up into teeth at an early stage of growth, sometimes remaining poroid, especially toward the margin of the pileus; spores white, smooth, oblong, curved, 2–2.6 x 7–8 μ.

Growing on old logs. September to December. Common.

The following group of characters will usually identify the species: the semi-resupinate habit of growth, whitish or tan-colored pileus, and the rather long tubes with large mouths, soon breaking up into teeth. *P. molliusculus* was named by Berkeley from specimens sent to him from Ohio by Lea. Morgan's determination of *P. molliusculus* was an error, his plants belonging to *P. pubescens* Schum. ex. Fries. Kellerman repeated the error in distributing *P. molliusculus* in his 'Ohio Fungi Fascicles.' For illustration see Hard, Mushrooms *f. 841.*

10. P. semipileatus Peck, Ann. Rept. N. Y. State Mus. **34**: 43. 1881.

Plants resupinate or effused-reflexed, rarely strictly sessile; pileus dimidiate or elongate, 0–1.5 x 0.7–3.5 x 0.1–0.5 cm., soft and spongy when fresh, rigid when dry, white, yellowish, or reddish brown, slightly tomentose to glabrous, azonate, margin thin, acute; context whitish, soft when fresh, firm when dry, 1–4 mm. thick; tubes less than 2 mm. long, the mouths white, greenish or somewhat violaceous, angular, minute, averaging 4–6 to a mm., the walls entire; spores white, smooth, oblong, curved, 1 x 3–4 μ.

On old limbs on the ground. September to December. Rare.

Easily recognized by the minute pores, the semi-resupinate habit of growth, and the often violet tinted hymenium. There is no previous record of the plant occurring in Ohio. Collections were made at Oxford, in 1911, for the first time.

11. P. caesius Schrad. ex Fries, Syst. Myc. **1**: 360. 1821.

Boletus caesius Schrad. Spic. Fl. Ger. 167. 1794.

Pileus sessile or effused-reflexed, dimidiate, 1–3.5 x 2–6 x 0.3–2 cm., soft and spongy when fresh, rigid when dry, whitish to cinereous, often with a bluish tinge, distinctly villous or tomentose especially behind; azonate, margin thin and acute; context white, soft, spongy and full of water when fresh, friable when dry, 0.3–1 cm. thick; tubes 2–7 mm. long, mouths white, pallid, or bluish gray, angular, averaging 3–5 to a mm., the

walls thin and usually lacerate; spores minute, white, smooth, cylindrical, sometimes curved, 1.2–1.5 x 4.7–5.2 μ.

On dead wood of deciduous and coniferous trees. October to December. Rare.

The bluish color of the pileus and hymenium is so often wanting that other characters must frequently be used in the identification of the plant. The slender tubes, usually longer than or as long as the thickness of the context, is apparently a rather constant character of the plant. The villous or tomentose pileus separates it from *P. chioneus* Fries and *P. lacteus* Fries and these are the only species with which it is likely to be confused.

12. P. chioneus Fries, Syst. Myc. 1: 359. 1821.

Pileus sessile, dimidiate, 1–3 x 2–5 x 0.5–3 cm., soft and spongy when fresh, rigid when dry, whitish to grayish or yellowish, azonate, glabrous or with a slight strigose tomentum towards the base, sometimes covered with a thin grayish or yellowish pellicle that becomes more evident on drying; margin acute, sometimes inflexed on drying; context white, soft and spongy when fresh, fragile when dry, 0.3–2 cm. thick, azonate, with a sweet acid odor; tubes 1–8 mm. long, mouths white or yellowish, usually glistening, angular, averaging 3–4 to a mm., the walls thin but entire; spores white, smooth, oblong, slightly curved, 1–1.7 x 4–5 μ.

On dead wood. September to November.

From *P. galactinus* Berk. this plant is most easily separated by the oblong, curved spores. The usually glabrous pileus and the absence of bluish tints separates it from *P. caesius* Schrad. ex Fries. Whether it is distinct from *P. lacteus* Fries may well be doubted. The plant is much in dispute in Europe. Our plants have been described as *P. albellus* Peck.

13. P. lacteus Fries, Syst. Myc. 1: 359. 1821.

Pileus pure white, fleshy-fibrous, fragile, triangular, pubescent, azonate externally and internally, margin inflexed, acute; pores thin acute, dentate, becoming torn and labyrinthiform. Commonly small and thin but sometimes large and transversely elongate, often gibbous behind, becoming glabrate and uneven. (Adapted from Fries, Hymen. Eur. 546.)

On dead wood of deciduous trees. Rare.

Until quite recently this and the preceding species have been held to be quite distinct. Of late years the European mycologists are coming to believe that they cannot be regarded as distinct species. Murrill would separate them on the ground that *P. chioneus* always has a distinct cuticle which is entirely lacking in *P. lacteus*. The writer has endeavored to keep the plants distinct on the basis of the differences noted by Fries. If this proves unfeasible then the two must be united as one species under the name of *P. chioneus*, at least with reference to their occurrence in this country.

14. P. galactinus Berk., Hooker's Lond. Jour. Bot. 6: 321. 1847.

Pileus sessile, imbricate or single, dimidiate, 3–7 x 3–7 x 0.5–2 cm., soft and pliant when fresh, more or less watery, rigid and contorted on drying, white, grayish, or somewhat yellowish, tomentose to strigose-tomentose, especially at the base, becoming glabrous with age, azonate, margin thin and acute; context white or pallid, watery and spongy when fresh, with a distinct sweet acid odor, firm when dry, sometimes more or less duplex, 3–8 mm. thick; tubes 2–7 mm. long, mouths white to bay, often glistening, circular to angular or sinuous, minute, averaging about 6 to a mm.; spores white, smooth, ellipsoid, 2–2.5 x 3.5–4 μ, uninucleate and with a very transparent wall.

Growing on dead wood of deciduous trees. August to November. Common.

The sweet acid odor mentioned in the description is a distinguishing character of all collections of this species. No mention is made of the odor in any published work to the writer's knowledge, except in Peck's description of *P. immitus* in which the odor is described as subacid. *P. immitus* is in all probability this plant. The odor is so constant that whenever it is noticed in connection with any minute-pored form of this section one can be sure that the plant belongs to this species.

All of the collections that I have referred to this species are watery when fresh, have a sweet acid odor, and when dried shrink much in size and often become much contorted. The context becomes thin and hard and takes on a resinous, dark brown or black color. This appearance may be uniform through

the context or the dark resinous color may be limited to a narrow line next to the hymenium or confined to two or three narrow zones in the context. It is difficult to distinguish these species with a white watery context and the writer's presentation of them may be open to criticism.

15. P. spumeus Sow. ex Fries, Syst. Myc. 1:358. 1821.

Boletus spumeus Sow. Col. Fig. Eng. Fungi *pl. 211.* 1797.

Pileus sessile, dimidiate, watery and fleshy-tough when fresh, firm when dry, 7–12 x 10–20 x 2–3 cm., much smaller on drying, appearing appressed-tomentose, white or grayish, somewhat yellowish or brownish on drying, azonate, margin rather thick but acute; context white, soft, spongy, and full of water, rather fragile on drying, more or less zonate, 1–3 cm. thick; tubes 0.5–1.5 cm. long, mouths white or yellowish on drying, angular, averaging 3 to a mm.; spores white, smooth, globose, or subglobose, 4.5–5.2 μ in diameter, distinctly uninucleate.

Growing on injured or diseased deciduous trees, especially *Ulmus* and *Acer*. October and November. Rare.

The plant is closely related to *P. delectans* Peck, with the same habitat and general appearance, but separated from that species by the smaller mouths of the tubes and by the distinctly uninucleate and more globose spores. The plants so referred do not agree with the figure given by Sowerby, nor with Fries' description. My plants were determined by Bresadola.

16. P. delectans Peck, Bull. Torr. Bot. Club 11:26. 1884.

Pileus sessile, sometimes imbricate, dimidiate in outline, 3–7 x 4–15 x 0.7–3 cm., rather spongy and watery when fresh, firm and rigid when dry, white or whitish, finely tomentose or glabrous, azonate, margin thin and acute; context white, in large specimens duplex, with a firm lower layer and a soft upper layer, in smaller specimens more uniform, 0.5–1.5 cm. thick; tubes 0.5–1.5 cm. long, mouths white, yellowish on drying, circular to angular, large, averaging 1–2 to a mm.; spores white, smooth, ellipsoid to globose, 4.5–5.5 x 6.5–8.5 μ.

On diseased or injured trunks of deciduous trees, especially *Acer;* sometimes on logs of *Fagus*. September to December. Frequent.

The species is separated from *P. spumeus* Sow. ex Fries by the larger tube mouths and the less globose spores that have

not been observed to be uninucleate as in that species. It is a large white fungus distinct from the other allied species in size, length of tubes, and habitat.

17. P. obtusus Berk. Ann. & Mag. Nat. Hist. I. **3**: 390. 1839.

Plants annual, sessile, sometimes imbricate; pileus dimidiate, convex or ungulate, 3–9 x 4–15 x 3–6 cm., somewhat spongy when fresh, firm, rigid, and very light in weight when dry, cinereous to yellowish or darker in herbarium specimens, hirtose-tomentose, rarely becoming glabrous, azonate, margin thick, obtuse; context white or whitish, spongy to corky, sometimes duplex, 1–3 cm. thick; tubes 1.5–3 cm. long, the mouths white, bay or brown on drying, circular to angular and sinuous, 1 mm. or more in diameter; spores (teste Murrill) globose, smooth, hyaline, 6–8 μ.

On trunks of diseased deciduous trees, especially *Quercus*. Rare.

Always easily recognized by the rounded and obtuse margin, and the long tubes with large mouths. Excellent illustrations are given by Spaulding (Ann. Rept. Mo. Bot. Gard. **16**: *pl. 13–19*).

18. P. guttulatus Peck, in Sacc. Syll. Fung. **6**: 106. 1888.

P. maculatus Peck, Ann. Rept. N. Y. State Mus. **26**: 69. 1874.

Pileus sessile, sometimes imbricate, dimidiate, 3–8 x 5–12 x 0.4–1.5 cm., soft and fleshy when fresh, firm and rigid when dry, white to yellowish or slightly brownish, glabrous, azonate or sometimes zonate on the margin, sometimes marked with rounded depressed spots, margin thin, acute; context white or pallid, soft and fleshy when fresh, soft-corky or friable when dry, 0.4–1 cm. thick; tubes 1–5 mm. long, the mouths white to yellowish or umbrinous, angular, averaging 4–5 to a mm.; spores white, smooth, oblong-ellipsoid, 2.5–3 x 3–5 μ. (Cf. Murrill, globose, smooth, hyaline, 5 μ in diameter.)

Growing on wood of coniferous trees. Rare.

The distinguishing character of the species is the presence of the round depressed spots on the pileus.

19. P. borealis Fries, Syst. Myc. **1**: 366. 1821.

Pileus sessile, dimidiate, sometimes with an attenuate base, 3–8 x 4–12 x 0.5–2.5 cm., somewhat watery and spongy when fresh, rigid when dry, white or yellowish, sometimes brownish, hispid to tomentose, azonate, margin thin and acute; context

white or yellowish, distinctly duplex, firm and fibrous below, soft and floccose above, 0.5–2 cm. thick; tubes 3–10 mm. long, the mouths white or yellowish, angular to irregular and uneven, rather large, averaging 2–3 to a mm.; spores (teste Murrill) ovoid, smooth, hyaline, 5–6 x 3–4 μ.

Growing only on trunks of coniferous trees. Rare.

The species is most easily separated from its allies by the size and habitat. For illustrations see Atkinson, Mushrooms *f. 9.*, Duggar, Fung. Dis. Plants *f. 228.*, and Atkinson, Cornell Univ. Agr. Exp. Sta. Bul. 193: *f. 63.*

20. P. Spraguei Berk. & Curt. Grevillea **1**: 50. 1872.

Plants annual, sessile or decurrent, sometimes imbricate; pileus dimidiate, 4–12 x 4–10 x 0.6–2 cm., fleshy-tough when fresh, rigid when dry, white or cinereous, appressed-tomentose or glabrous, azonate or somewhat zonate, margin thin or rather thick, acute, often blackening on drying; context white, watery, tough-fibrous when fresh, sometimes very hard when dry, zonate, 0.3–1.5 cm. thick, with a disagreeable odor in fresh specimens; tubes 0.3–1 cm. long, mouths white or discolored, circular or angular, averaging 3–4 to a mm.; spores (teste Murrill) ellipsoidal smooth, hyaline, 6 x 4 μ.

On dead wood of deciduous trees, especially on *Fagus*, *Quercus*, and *Castanea*. July to September. Common.

Fresh specimens are always easily distinguished by the very disagreeable odor. Dried plants are characteristically very hard and rigid, the context almost bony in texture.

21. P. zonalis Berk. Ann. & Mag. Nat. Hist. I. **10**: 375. 1842.

Plants annual, sessile, effused-reflexed, or entirely resupinate; pileus dimidiate or laterally confluent, 0–2.5 x 1–5 x 0.2–0.5 cm., fleshy and pliable when fresh, rigid and firm when dry, whitish to flesh-colored or isabelline, finely tomentose to glabrous, at first azonate but becoming zoned when mature, the margin at first thick, thin with age; context white, fibrous when fresh, hard and rigid when dry, 1–2 mm. thick; tubes 1–3 mm. long, the mouths usually more or less flesh-tinted when fresh, angular, averaging 4–5 to a mm., the walls thick and entire, very firm and rigid on drying; spores white, smooth, globose, 2.5–5 μ broad, with one large nucleus.

On old rotting logs, especially of *Liriodendron*. August to December. Not common.

The writer has collected this plant several times in the Miami valley, almost always on logs of *Liriodendron tulipifera*. The plant is usually entirely resupinate and has doubtless been described as a *Poria*, but good collections were made which showed beyond a doubt the pileate tendency of the plant. No disposition could be made of the plant until Dr. Murrill suggested that it might belong to *P. zonalis*. Later, specimens were sent to Rev. Bresadola who pronounced it that species and an opinion recently received from Mr. Lloyd expresses the same view. It is, however, quite different from the usual forms of that plant and the name is used with some apprehension. The plant is also abundant in Missouri where the writer has found the pileate forms to be much more common than in Ohio. *P. zonalis* has been supposed to be confined to the Gulf States in this country, although it is not surprising that semi-tropical forms found there should extend their range up the large river valleys to the north.

22. P. adustus Willd. ex Fries, Syst. Myc. 1: 363. 1821.
Boletus adustus Willd. Fl. Berol. 392. 1787.

Plants annual, sessile, effused-reflexed, or resupinate; pileus dimidiate, often imbricate, 1–6 x 2–7 x 0.2–0.4 cm., fleshy-tough when fresh, coriaceous or rigid when dry, white to cinereous or pale tan, fibrillose-tomentose to almost glabrous, zonate or azonate, the surface usually rough, margin thick and broadly sterile below when young, becoming thin when mature; context white or pallid, rather soft when fresh, corky or fibrous-corky when dry, 1–3.5 mm. thick; tubes not more than 1 mm. long, the mouths grayish black to black, angular, even, minute, averaging 5–7 to a mm.; spores white, smooth, oblong to oblong-ellipsoid, 2–2.5 x 3.8–4.3 μ.

On stumps and trunks of dead deciduous trees. August to December.

This species differs from *P. fumosus* Pers. ex Fries and *P. fragrans* Peck in the smaller size and the uniformly black hymenium.

23. P. fragrans Peck, Rept. N. Y. State Museum **30**: 45. 1879.

Plants annual, sessile or effused-reflexed; pileus dimidiate, imbricate, 2–8 x 4–10 x 0.5–2 cm., fleshy-tough when fresh, firm and rigid when dry, cinereous to reddish gray, finely tomentose to almost glabrous, subzonate or azonate, the margin thin and acute; context whitish or pallid, tough when fresh, soft-corky when dry, 4–8 mm. thick, with a sweet anise-like odor that persists in dried plants; hymenium sometimes separated from the context by a narrow, dark-colored line; tubes less than 4 mm. long, the mouths whitish or somewhat smoke-colored, blackish when bruised, angular, the dissepiments becoming dentate and the mouths unequal in size, averaging 3–4 to a mm.; spores (teste Murrill) white, globose to ovoid, smooth, 5–6 μ in diameter.

On stumps and trunks, especially of *Ulmus*. Frequent.

The distinguishing characters of this species are the fragrant odor and the unequal and irregular pores—characters which separate it from *P. adustus* and *P. fumosus*. The name *P. puberula* Berk. & Curtis is sometimes applied to this plant.

24. P. fumosus Pers. ex Fries, Syst. Myc. **1**: 367. 1821.
Boletus fumosus Pers. Syn. Fung. 530. 1801.

Plants annual, sessile or effused-reflexed; pileus dimidiate, often imbricate, 2–7 x 3–8.5 x 0.3–2 cm., somewhat fleshy-tough when fresh, firm and rigid when dry, grayish to very pale tan-colored, finely tomentose, subzonate or azonate, margin thin and acute; context white to light umber, soft corky when fresh, corky when dry, 0.3–2 cm. thick, with a rather disagreeable odor; hymenium separated from the context by a distinct, narrow, dark-colored line; tubes short, not more than 3 mm. long, the mouths whitish or smoky, blackish when bruised, circular to somewhat angular but thick-walled and entire, averaging 4–6 to a mm., spores white, smooth, elliptical to subcylindrical, 2.6–4 x 5.3–7.2 μ.

Growing on dead wood of deciduous trees. October to December. Frequent.

Distinguished from *P. fragrans* Peck by the more circular and entire tube mouths and, in our plants at least, by the absence of the fragrant, anise-like odor. The odor is disagreeable in

the fresh plants but disappears on drying. Bresadola ascribes a subanise odor to the plant at times. The plants are, however, closely related and one may expect to find intermediate forms that are difficult to refer to either species. Thin, semi-resupinate forms are often scarcely distinguishable from *P. adustus* Willd. ex Fries. The plant is illustrated by Bresadola (Fungi Trident. *pl. 135*).

25. P. robiniophila (Murr.) Overholts, n. comb.

Trametes robiniophila Murr. N. Am. Flora **9**: 42. 1907.

Plants annual, sessile, rarely imbricate; pileus dimidiate, fleshy-tough or somewhat coriaceous when fresh, firm and rigid when dry, 3.5–10 x 4–15 x 1–4 cm., white to cinereous or yellowish, finely tomentose to glabrous, azonate or rarely subzonate or concentrically sulcate in large specimens, margin at first thick and obtuse, becoming thin and acute when mature; context white, fleshy-tough when fresh, soft and punky when dry, 0.5–3 cm. thick, usually with a sweet anise-like odor developing in herbarium specimens; tubes 0.3–1 cm. long, mouths white, often bay or brownish in dried plants, circular to angular, averaging 4–6 to a mm., the walls thick and entire; spores white, smooth, ovoid to subglobose, 5.5–7 x 7–8.5 μ.

On deciduous trees, especially *Robinia*, *Celtis*, and *Acer*. August to December. Common.

Dried plants are characterized by the tough, punky context and the sweet odor, as well as by the large size of the plant, the long tubes, the minute mouths, and the habitat. The plant was first described as a *Trametes* but it appears to belong rather to *Polyporus*.

26. P. betulinus Bull. ex Fries, Syst. Myc. **1**: 358. 1821.

Boletus betulinus Bull. Herb. Fr. *pl. 312*. 1786.

Pileus sessile or attached by a prominent lateral umbo, dimidiate to circular in outline, 3–9 x 3–15 x 1–5 cm., somewhat fleshy when young, firm and rigid when dry, glabrous, azonate, smooth, covered with a thin pellicle, margin more or less incurved, with a wide sterile band on the lower surface; context white, somewhat fleshy when fresh, soft-corky when dry, 1–3.5 cm. thick; tubes 3–8 mm. long, mouths white, circular to angular, averaging 3–4 to a mm.; hymenium at times covered by projecting setae, sometimes as much as 2 mm. long; tubes separat-

ing in a smooth layer from the context; spores (teste Murrill) white, cylindrical, curved, 4–5 μ long.

Growing only on *Betula*. Not common.

Always easily recognized by the habitat, the smooth, pelliculose surface and the inrolled, broadly sterile margin of the pileus. Good illustrations are given by Freeman (Minn. Plant Diseases *f. 126*), Hard (Mushrooms *f. 337*), White (Hymen. Conn. *pl. 37*), and Kellerman (Ohio Myc. Bul. 10: *f. 43*).

27. P. volvatus Peck, Rept. N. Y. State Mus. **27**: 98. 1875.

Plants annual, sessile or attached by a stem-like base; pileus globose or compressed-globose in form, 1–5.5 cm. broad, 1–3.5 cm. thick, somewhat coriaceous-corky when fresh, hard and firm when dry, somewhat encrusted, whitish or yellowish, sometimes tinged with red, glabrous, azonate, margin thick and rounded, extending downward and backward and forming a veil-like covering over the hymenium; context white or light colored, soft-corky, 0.2–1 cm. thick; tubes 2–5 mm. long, the mouths whitish to brownish, circular, averaging 3–4 to a mm.; the covering over the hymenium ruptures in from one to three places and allows the escape of the spores; spores (teste Peck) flesh-colored, elliptical, 5 x 7.5–9 μ.

On dead wood of coniferous trees. Rare.

An aberrant form easily recognized by the veil-like covering of the hymenium. This is persistent, being coriaceous in texture and as much as 1 mm. thick. Peck's illustration (Rept. N. Y. State Mus. **27**. *pl. 2. f. 3–6*) gives some idea as to the general form of the plant; Hard's (Mushrooms *f. 340*) is not much better. von Schrenk gives a good illustration (U. S. Dept. Agr., Div. Veg. Path. Bul. 25: *pl. 1. f. 2*).

28. P. distortus Schw. ex Fries, Elench. Fung. **1**: 79. 1828.

Boletus distortus Schw. Syn. Fung. Car. 97. 1822. *Polyporus abortivus* Peck, Bot. Gaz. **6**: 274. 1881.

Plants stipitate or substipitate, variable in form and size, sometimes with a distinct, well developed, centrally placed stipe, sometimes the whole plant distorted and the stipe rudimentary, often almost the entire surface of such forms covered with the tubes; pileus circular to irregular in outline, fleshy-tough when fresh, firm and coriaceous when dry, variable in color, whitish, grayish, tan-colored, rufescent, or brownish, vil-

lous-tomentose, soft to the touch, azonate, margin thin and acute or thick and obtuse; context white or whitish, with a firm corky layer next to the hymenium and a lighter colored, softer layer above, the whole 0.2–1 cm. thick; tubes in well developed specimens 1–6 mm. long, whitish or rufescent when bruised, mouths angular to dædaloid and irregular, averaging 1–3 to a mm.; stipe central, lateral, or wanting, rarely well developed and up to 6 cm. long, more often rudimentary and tubercular, clothed like the pileus, soft on the outside and firm within; spores white, smooth, subglobose, 5.5–8.5 μ in diameter; conidial (?) spores sometimes present, white, smooth, ovoid to elliptical, 3.3–4.2 x 5.2–7.8 μ.

Usually growing about stumps and probably always attached to buried wood. Common.

Well developed specimens of this plant will be easily recognized by the duplex context and the soft, villous pileus; abnormal specimens by their distorted appearance. The duplex context is always more easily recognized in dried specimens. According to Lloyd our plant is identical with *P. rufescens* Fries of Europe. See Lloyd, Syn. Stip. Polyp. *f. 458.*, for illustration of one form of the distorted plant.

29. P. pocula Schw. ex Berk. & Curt. Proc. Am. Acad. Arts Sci. **4**: 122. 1858.

Sphaeria pocula Schw. Jour. Acad. Nat. Sci. Phil. **5**: 7. 1825. *Enslinia pocula* Schw. ex Fries, Summ. Veg. Scand. **2**: 399. 1849.

Pileus short-stipitate, pendant from dead branches, circular in outline, 1–5 mm. in diameter, 1–3 mm. thick, coriaceous when fresh, rigid when dry, whitish to brown in color, pruinose or mealy, azonate; context coriaceous when fresh, hard when dry, less than 1 mm. thick; tubes not more than 0.5 mm. long, mouths at first appearing pruinose, whitish or brownish, circular, very minute, averaging 5–6 to a mm.; stipe dorsally attached, concolorous with and expanding into the pileus, pruinose, not more than 5 mm. long; spores (teste Murrill) globose, smooth, hyaline, 4 μ in diameter.

On dead branches, especially of *Quercus* and *Castanea*. Rare.

This is the smallest known polypore and easily identified by its size and habit of growth. It was first decribed as an asco-

mycete (*Sphaeria*) by Schweinitz and later transferred to the genus *Enslinia* (*Pyrenomycetes*) by Fries. Excellent illustrations are given by Lloyd (Myc. Notes, Polyp. Issue 3: *f. 369–70;* Syn. Stip. Polyp. *f. 443*).

30. P. brumalis Pers. ex Fries, Syst. Myc. 1: 348. 1821. *Boletus brumalis* Pers. Neues Mag. Bot. 1: 107. 1794.

Pileus stipitate, circular in outline, sometimes somewhat umbilicate in the center, 1.5–5 cm. broad, 0.2–0.4 cm. thick, fleshy-tough when fresh, rigid when dry, varying in color from yellowish brown to dark brown or almost black, minutely hispid to glabrous, rarely slightly squamulose, usually azonate but at times distinctly zoned, margin thin and entire, involute when young and incurved on drying; context white or pallid, soft-fibrous when fresh, firm when dry, 2 mm. or less thick; tubes 1–3 mm. long, usually slightly decurrent, the mouths white or whitish, at first circular and thick walled, later angular and the dissepiments thinner, averaging 2–3 to a mm.; stipe central or subcentral, simple, cylindrical, grayish or brownish, minutely hispid or glabrous, 2–3 cm. long, 0.2–0.3 cm. thick; spores white, oblong, sometimes slightly curved at one end, smooth, 2.5 x 9 μ.

Growing on dead wood in the fall and early winter. Common.

P. brumalis and *P. arcularius* are closely related species that are not always easy to separate. In general the forms occurring in the early spring and summer are likely to be *P. arcularius*, while those found in autumn and often late in winter are more likely to be *P. brumalis*. Hard (Mushrooms *f. 335*) gives a good illustration of the plant.

31. P. arcularius Batsch. ex Fries, Syst. Myc. 1: 342. 1821. *Boletus arcularius* Batsch. El. Fung. 97. 1783. *P. arculariformis* Murrill, Torreya 4: 151. 1904.

Pileus stipitate, circular in outline, convex to umbilicate, sometimes infundibuliform, 1–8 cm. broad, 1–4 mm. thick, fleshy-tough or coriaceous when fresh, rigid when dry, golden brown to dark brown, usually more or less squamulose, azonate, the margin usually distinctly ciliate, involute on drying; context white or pallid, fibrous-fleshy when fresh, compact-fibrous when dry, less than 2 mm. thick; tubes 1–2 mm. long, often decurrent, the mouths white, discolored on drying, angular and

often radially elongate, averaging 2 to a mm. in transverse direction and about 1 to a mm. in axial direction; stipe central or subcentral, concolorous with the pileus, fuscous-squamulose to glabrous above, often hispid at the base, 2–6 cm. long, 2–4 mm. thick; spores white, smooth, elliptical-cylindrical, usually 2–3 guttulate, 2–3 x 6–8.5 μ.

On dead wood. Common.

This species is much more common than the preceding and is distinguished from it by the lighter colored pileus, the ciliate margin, the hispid stipe base, and the larger and more alveolar tubes. Is is usually found in the spring and early summer. A small form of it with the pileus not more than 1 cm. in diameter is especially common on twigs and bits of wood during the late spring and early summer. Murrill regards this form as a distinct species and has named it *P. arculariformis* (Torreya 4: 151). It is here maintained as a form of *P. arcularius*. This species is well represented by Hard (Mushrooms *f. 336*).

32. P. pennsylvanicus Sumstine, Jour. Myc. **13**: 137. 1907.

Pileus stipitate, circular in outline, depressed, sometimes umbilicate or somewhat infundibuliform, 4–6.5 cm. in diameter, 0.2–0.5 cm. thick, fleshy-tough, pale tan or ochraceous buff in color, with a thin cuticle, glabrous, azonate, margin thin and acute; context white, soft and watery when fresh, with a sweet acid odor, rather fragile when dry, 2–4 mm. thick; tubes white at first, discolored on drying, long decurrent on the stipe, 2–4 mm. long, mouths angular, thin walled, large, somewhat longer in the radial direction, 1–2 mm. long, 0.5 to 1 mm. wide; stipe central or excentric, whitish, glabrous, expanding above, 2–3 cm. long, 0.4–1 cm. thick; spores white, smooth, oblong-elliptical or fusoid, 4.2–5.7 x 10–14 μ, often once to several times guttulate.

Growing on old logs in July and August. Frequent.

The above description is drawn from notes and specimens from two collections made at Oxford, Ohio, one in August, 1910, and the other in July, 1911. The odor of the fresh plant is described by the author as "nitrous". The large angular pores ally the species with *P. arcularius* Batsch. ex Fries and with *Favolus canadensis* Klotzsch. From the former it is easily separated by the much larger spores and from the latter by

the well developed stipe with the decurrent tubes, the usually umbilicate pileus, and the friable context when dry. Possibly it should be referred to *P. Rostkowii* Fr. or to *P. pallidus* Schulz. & Kalchbr., both of which some regard as being small scaleless forms of *P. squamosus* Huds. ex Fries. The spores agree well with those of *P. squamosus*, but although it can be shown to be related to that species, it is worthy of a distinct name.

33. P. squamosus Huds. ex Fries, Syst. Myc. 1: 343. 1821.
Boletus squamosus Huds. Fl. Angl. 626. 1798. [2nd ed.]

Pileus short-stipitate or almost sessile, dimidiate to reniform in outline, 6–25 cm. in diameter, 0.5–4 cm. thick, fleshy when fresh, firm and rigid when dry, whitish to dingy yellowish or brownish, clothed, especially toward the center, with large, appressed, brownish scales often concentrically arranged, azonate, margin thin and acute; context white, tough, soft-corky when dry, 0.5–3.5 cm. thick; tubes 2–8 mm. long, decurrent, the mouths white or yellowish, large and angular, 1–2.5 mm. in diameter; stipe lateral, often rudimentary, black at the base, reticulate above by the decurrent pores, 1–5 cm. long, 1 cm. or more thick.

Growing on injured or diseased deciduous trees. Rare.

Lloyd gives the spores as "oblong, 5–6 x 12–15 μ, hyaline, smooth." Easily recognized by the large pores and the large, appressed, brownish scales. The plant is well illustrated by Bresadola (Fung. Trident. *pl. 133*), Freeman (Minn. Pl. Diseas. *f. 125*), Lloyd (Photograph. *pl. 5*), and Hard (Mushrooms *f. 325*).

34. P. picipes Fries, Syst. Myc. 1: 353. 1821.
P. fissus Berk. Hooker's Lond. Jour. Bot. **6**: 318. 1847.

Pileus stipitate, circular to reniform in outline, convex or plane, when older usually becoming depressed or somewhat infundibuliform, 4–20 cm. broad, 0.1–0.8 cm. thick, tough and leathery when fresh, very rigid and brittle when dry, sometimes yellowish brown but usually dark chestnut-brown to reddish brown, usually lighter in color towards the margin, azonate, margin very thin, usually wavy and often lobed; context white to somewhat ochraceous, leathery when fresh, firm when dry, 1–7 mm. thick; tubes not more than 2 mm. long, decurrent on

the stipe, the mouths white to brownish in color, circular to angular, very minute, invisible to the unaided eye, averaging 5–7 to a mm.; stipe central to lateral, dark brown or black on the lower half, glabrous, 1–6 cm. long, 0.4–1.5 cm. thick.

On stumps and logs late in autumn. Common.

The combination of black stipe base and minute pores characterizes this and the next species. The two are separated mainly on point of size. Murrill describes this plant under the name of *P. fissus* Berk., which was originally described from specimens collected in Ohio. Patouillard (Tab. Fung. No. 136) says the spores are ovoid. Lloyd now considers this plant to be a form of *P. varius* Fries, of Europe. A good illustration of our plant will be found in Hard, Mushrooms *f. 319*.

35. P. elegans Bull. ex Fries, Epicr. Syst. Myc. 440. 1838.
Boletus elegans Bull. Herb. Fr. *pl. 46*. 1780.

Pileus stipitate, circular to reniform in outline, convexo-plane or depressed, 1.5–7 cm. in diameter, 0.2–1 cm. thick, leathery when fresh, rigid and firm when dry, pale ochraceous to dull orange-color, pruinose to glabrous, azonate, the margin rather thin, often radiate-striate, even or undulate; context white to light ochraceous, tough when fresh, soft corky when dry, 1–6 mm. thick; tubes 1–3 mm. long, decurrent on the stipe, the mouth whitish to pallid, circular to angular, averaging 4–5 to a mm.; stipe central, excentric or lateral, slender, black at the base, light colored above, pruinose or glabrous, 1-8 cm. long, 0.2–0.6 cm. thick.

On dead wood late in autumn. Not common.

Spores were not obtained from the writer's specimens. Murrill gives them as "oblong, smooth, hyaline, 7–8 x 3–3.5μ." The species is closely related to *P. picipes* Fries but is separated from it by the smaller size and the uniform ochraceous color of the pileus that never takes on the darker chestnut shades assumed by *P. picipes*. Bulliard (Herb. Fr. *pl. 124*) gives an excellent illustration of the plant under the name of *Boletus nummularius* Bull.

36. P. radicatus Schw. Trans. Am. Phil. Soc. II. **4**: 155. 1832.

P. Morgani Peck, Ann. Rept. N. Y. State Mus. **32**: 34. 1879.

Pileus stipitate, circular in outline, 3.5–20 cm. broad, 0.3–0.8

cm. thick, fleshy or fleshy-tough when fresh, more or less friable when dry, yellowish brown or darker, finely tomentose or fibrillose-scaly, often becoming glabrous, azonate; margin thin and acute, often involute on drying; context white or light yellowish, soft and spongy, 2–6 mm. thick; tubes 1–5 mm. long, decurrent on the stipe, the mouths white or brownish on drying, circular to angular and irregular, averaging 2–3 to a mm.; stipe central, simple or rarely branching once or twice, yellowish or brownish, prolonged below into a long, black, rooting base, velvety or rough-squamulose above, 6–15 cm. long, 0.5 to 2 cm. thick; spores white, smooth, ovoid-elliptical, 6–8 x 12–15 μ.

Growing on the ground, sometimes around stumps, and probably attached to buried wood. Common.

This plant is always easily recognized by the black and radicating base of the stem. The type specimens of *P. Morgani* Peck were collected in Ohio by Morgan. For illustrations see Hard, Mushrooms *f. 329.*, Lloyd, Syn. Sec. Ovinus *f.* 508; Syn. Stip. Polyp. *f. 465.*, and Ohio Myc. Bull. 11: *f. 46.*

37. P. flavovirens Berk. & Curt. Grev. 1: 38. 1872.

Pileus stipitate, circular to irregular in outline, 4–10 cm. broad, 0.3–0.8 cm. thick, soft and fleshy when fresh, rigid but friable when dry, yellowish green or yellowish brown in color, the surface often cracked and areolate and the flesh showing yellowish in the cracks, slightly tomentose or glabrous, azonate, the margin thin and acute; context white or yellow, fleshy when fresh, soft and friable when dry, 1–4 mm. thick; tubes 1–5 mm. long, decurrent on the stipe, the mouths white or yellowish, sometimes reddish on drying, circular to angular, averaging 1–3 to a mm.; stipe simple or branched, usually excentric but sometimes central, often irregular in form, whitish or yellowish in color, 3–6 cm. long, 1–1.5 cm. thick; spores white, smooth, globose, or subglobose, 3–4.7 μ in diameter.

Growing on the ground in deciduous woods. Frequent in July and August.

A species easily recognized by the color of the pileus. The plant is fairly well represented by Hard (Mushrooms *f. 327*), and by Lloyd (Syn. Sect. Ovinus *f. 501*). According to Lloyd *P. cristatus* Pers. of Europe is not different from our plant. Murrill lists it under the name of *Grifola poripes* Fries ex Murr.

38. P. umbellatus Pers. ex Fries, Syst. Myc. 1: 354. 1821.
Boletus umbellatus Pers. Syn. Fung. 519. 1801.

Plants stipitate, 7–20 cm. in diameter, the stipe branching repeatedly and giving rise to many centrally attached pileoli which are circular in outline, 1–4 cm. broad, less than 5 mm. thick, fleshy in texture when fresh, rigid when dry, whitish to smoky brown in color, fibrillose or glabrous, azonate; margin thin, acute, entire; context white, fleshy or fleshy-tough, rather brittle when dry, usually not more than 1 mm. thick; tubes less than 2 mm. long, decurrent on the stipe branches, the mouths white, angular, averaging 2–4 to a mm.; stipe compound, the branches cylindrical in form, central or subcentral, white, usually entirely covered with the decurrent tubes; spores white, oblong-elliptic, smooth, 2.3–3.5 x 7–9.4 μ.

Growing about the bases of stumps or trees, especially of *Quercus*. Rare.

Easily distinguished from its allies by the more regular and cylindrical stipe branches, the small and centrally attached pilei which are more or less circular in outline, and by the oblong-elliptic spores. Murrill describes it as *Grifola ramosissima* Scop. ex Murr. The plant is well illustrated by Lloyd (Syn. Stip. Polyp. *f. 450*), Hard (Mushrooms *f. 320*), and Atkinson (Mushrooms *f. 183*).

39. P. frondosus Dicks. ex Fries, Syst. Myc. 1: 355. 1821.
Boletus frondosus Dickson, Fasc. Pl. Crypt. Brit. 1: 18. 1785.

Plant stipitate, the stipe many times branching and giving rise to numerous overlapping pileoli, the whole plant forming a more or less globose mass often as much as 40 cm. in diameter; pileoli flabelliform or spathulate in outline, 2–7 cm. broad, 2–5 mm. thick, fleshy-tough when fresh, rigid when dry, grayish to mouse-colored, glabrous or minutely tomentose, azonate, the margin thin and acute; context white or whitish, fleshy-tough when fresh, fragile when dry, not more than 2 mm. thick; tubes 2–3 mm. long, decurrent on the stipe, the mouths white, angular or irregular, averaging 1–3 to a mm.; stipe compound, short and thick; spores white, smooth, ovoid to elliptical, 4.5–6 x 6–9 μ.

Usually found at the bases of trees or stumps, preferably of *Quercus* and *Ulmus*. Common in late fall.

From *P. Berkeleyi* Fries and *P. giganteus* Fries this species is separated by the numerous small pileoli which in those species are large and few in number. The irregular stipe-branches and the more spathulate pileoli separate it from *P. umbellatus* Fries in which the stipe branches are cylindrical and the pileoli centrally attached and consequently more nearly circular in outline. The plant is illustrated in Atkinson, Mushrooms *f. 181–82.*, Hard, Mushrooms *f. 321.*, and McIlvaine, Am. Fungi *pl. 128.*

40. P. giganteus Pers. ex Fries, Syst. Myc. **1**: 356. 1821. *Boletus giganteus* Pers. Syn. Fung. 521. 1801. *Grifola Sumstinei* Murrill, Bull. Torr. Club **31**: 335. 1904.

Plants composed of a few broad pileoli, 6–15 cm. in diameter and less than 0.5 cm. thick, dimidiate to flabelliform or spathulate in outline, fleshy-fibrous when fresh, more rigid when dry, grayish to brown, often black when dried—especially on the margin—,usually somewhat tomentose or fibrillose, azonate or subzonate, margin very thin and acute, often lobed, involute on drying; context white, fibrous, tough, 1–3 mm. thick; tubes 1–3 mm. long, at first white but blackish where bruised and on drying, the mouth angular to irregular, often torn, averaging 5–7 to a mm.; stipe short and thick; spores white, smooth, globose, 4–6 μ broad.

Growing on the ground around stumps. Frequent.

Separated from *P. Berkeleyi* Fries by the smooth spores; from *P. umbellatus* Pers. ex Fries, and *P. frondosus* Fries, by the much larger and fewer pileoli, and distinct from all of these in the blackening of the margin or of the entire pileus and hymenium when bruised or in drying. In the 'North American Flora' it is described under the name of *Grifola Sumstinei* Murr. In this country it has always been held to be the same as the European plant *P. giganteus* Fries, and European specimens recently received from Bresadola confirm this view. A very good illustration will be found in Bresadola, Fungi Tridenti *pl. 134.*, and in Boudier, Ic. Myc. **1**: *pl. 153.* To the writer's knowledge it has not been illustrated in American mycology.

41. P. Berkeleyi Fries, Nov. Sym. 40. 1851.
P. anax Berk. Grev. **12**: 37. 1882.

Pileus stipitate, the stipe sometimes branching and giving

rise to from 2 to 4 pileoli, sometimes simple with but one large pileus; pileoli fleshy-tough when fresh, becoming rigid on drying, more or less circular in outline, 6–15 cm. broad, 0.3–1.5 cm. thick, light colored, whitish to yellowish, slightly tomentose or glabrous, azonate or obscurely zoned; margin rather thin, often lobed; context white, fleshy-tough, fragile when dry, 0.3–2 cm. thick; tubes 2–8 mm. long, decurrent on the stipe; mouths white or whitish, large and irregular, averaging 0.5–2 mm. in diameter; stipe short and thick, more or less tubercular, whitish in color, 4–7 cm. long, 3–5 cm. thick; spores white, minutely echinulate, globose, 5.6–8.4 μ in diameter.

Growing at the bases of trees and stumps, especially of *Quercus*. Frequent.

This is one of the largest of our species and is easily distinguished from all of its allies by the echinulate spores. Morgan's description of *P. anax* Berk. applies to *P. frondosus* Fries and not to *P. Berkeleyi* for which *P. anax* is a synonym. (See Lloyd, Mycological Notes 27: 341–342.) The plant is well represented by the following illustrations: Lloyd, Photogr. *pl. 9–10;* Myc. Notes Polyp. Iss. 3: *f. 362–63.*, and Hard, Mushrooms *f. 323* and *pl. 45.*

42. P. sulphureus Bull. ex Fries, Syst. Myc. **1**: 357. 1821. *Boletus sulphureus* Bull. Herb. Fr. *pl. 429.* 1788. *P. cincinnatus* Morgan, Jour. Cinc. Soc. Nat. Hist. **8**: 97. 1885.

Plants annual, often attenuate at the base and appearing substipitate, imbricate; pileus dimidiate to flabelliform in outline, 5–20 x 4–12 x 0.5–2.5 cm., fleshy and watery when young, becoming firm when old, yellowish to bright orange-colored, sometimes fading with age, finely tomentose to glabrous, azonate or with broad colored zones, the margin thin and acute, sometimes lobed; context white or light yellow, fleshy when fresh, rather soft and friable when dry, 0.4–2 cm. thick; tubes 1–4 mm. long, the mouths bright sulphur-yellow, sometimes whitish or dull yellow with age or on drying, angular, averaging 2–4 to a mm.; spores white, smooth, ovoid to subglobose, 4–5 x 5.5–7 μ.

Growing on trunks and stumps of deciduous trees. Common.

Specimens usually change color on drying and most of the red color of the pileus is lost. The bright yellow of the hyme-

nium may or may not persist. The best colored representation of the fungus is that given by Rostkowius in Sturm, Deutschl. Flora 4: *pl. 20.* The plant is widely distributed and well known and has figured largely in American mycology. The following illustrations will aid in determinations: Atkinson, Mushrooms *f. 184–85.*, Duggar, Fung. Dis. Pl. *f. 226.*, Hard, Mushrooms *f. 326.*, and von Schrenk, U. S. Dept. Agr., Div. Veg. Path. Bul. 25: *pl. 11. f. 1–4.*

43. P. Pilotae Schw. Trans. Am. Phil. Soc. II. 4: 157. 1832.

P. hypococcineus Berk. Lond. Jour. Bot. 6:319. 1847.

Plants annual, sessile; pileus dimidiate, often subungulate, 5–12 x 6–15 x 1–5 cm., soft coriaceous or corky, buff or orange-colored, becoming whitish on drying, minutely tomentose or glabrous, azonate, margin usually obtuse; context pale buff, becoming carneous when dry, fibrous, sometimes very hard when dry, strongly zonate, 0.7–2 cm. thick; tubes 0.5–2 cm. long, the mouths orange-colored, becoming brownish on drying, angular, averaging 3–5 to a mm.; spores (teste Murrill) smooth, hyaline, 3–4 x 2–3 μ.

On dead wood of *Quercus* and *Castanea.* Rare.

Easily distinguished from other species with a predominance of red or orange colors by the thick pileus and the long tubes. The plant is said to emit a strong odor when growing. The type specimens of *P. hypococcineus* Berk. were collected in Ohio by Lea. *P. castanophilus* Atk., described from North Carolina, is said to be the same plant.

44. P. sanguineus L. ex Fries, Syst. Myc. 1: 371. 1821.

Boletus sanguineus L. Sp. Plant. 1646. 1762. [2nd ed.]

Plants annual, sessile; pileus dimidiate to flabelliform, 2–5 x 2–8 x 0.2–0.5 cm., coriaceous, bright red, finely tomentose to glabrous, often zonate, the margin very thin and acute; context red or yellowish red, soft and floccose, scarcely more than 2 mm. thick; tubes 0.5–1.5 mm. long, the mouths red, more or less angular or circular when young, averaging 2–4 to a mm.; pileus often attached by an attenuate base and then appearing substipitate.

On dead wood of deciduous trees. September to December. Rare.

The species is distinguished from the following one by the much thinner pileus and the marked tendency to appear substipitate. Otherwise it scarcely differs, and intermediate forms are found that are difficult to place satisfactorily It is usually considered to be a southern species, but Hard reports finding it in Ohio. His specimens were determined by Peck.

45. P. cinnabarinus Jacq. ex Fries, Syst. Myc. 1: 371. 1821.
Boletus cinnabarinus Jacq. Fl. Austr. 4: 2. 1776.

Plants annual, rarely reviving, sessile or effused-reflexed; pileus dimidiate or reniform, 2–6 x 2–10 x 0.5–2 cm., tough and leathery when fresh, more rigid when dry, orange-colored to cinnabar-red, often becoming paler or almost white with age, compactly tomentose or glabrous, usually azonate, margin thin or thick, acute; context red or yellowish red, floccose-fibrous to soft-corky, always zonate, 0.4–1.5 cm. thick; tubes 1–4 mm. long, the mouths cinnabar-red, circular then angular and sometimes somewhat sinuous, averaging 2–4 to a mm.; spores white, smooth, oblong, 2–2.5 x 4.5–5.5 μ.

On dead wood of all kinds. September to December. Common.

The prevailing deep red color of both pileus and hymenium separates this species from all others of the genus except *P. sanguineus* Fries, from which this species differs only in being thicker and in having the context more strongly zoned. *P. cinnabarinus* is a northern species and much more common in Ohio than is *P. sanguineus*.

46. P. resinosus Schrad. ex Fries, Syst. Myc. 1: 361. 1821.
Boletus resinosus Schrad. Spic. Fl. Ger. 171. 1794.

Plants annual, sessile or decurrent, more or less imbricate; pileus dimidiate, 5–15 x 7–25 x 0.8–2.5 cm., somewhat fleshy and full of water when young, firmer when mature and soft-corky on drying, velvety-tomentose to glabrous, sulcate or with a few broad, colored zones, margin at first thick and somewhat obtuse, becoming thinner and acute; context pallid to light brown, fleshy and watery when young, soft-corky when dry, 0.5–2 cm. thick; tubes 1–6 mm. long, the mouths white to pallid, changing to a darker color on drying, circular to angular, averaging 4–6 to a mm.; spores white, smooth, cylindrical, curved, 1.2–2 x 5–6.3 μ.

On old logs and stumps in October and November. Common.

Distinguished by the brown pileus and the light brown, almost whitish context. For illustration see Hard, Mushrooms *f. 331.*

47. **P. nidulans** Fries, Syst. Myc. 1:362. 1821.

Plants sessile or effused-reflexed; pileus dimidiate, 1.5–6 x 2–8 x 0.5–2 cm., very soft, spongy, and full of water when fresh, firm and friable when dry, umber to cinnamon or tawny brown, finely villous-tomentose to glabrous, azonate, margin thin and acute, purplish or reddish where bruised; context concolorous with the pileus, sometimes with a darker layer next to the hymenium, soft and watery when fresh, cheesy and friable when dry, 2–8 mm. thick; tubes 2–7 mm. long, mouths hoary when young, yellowish or reddish brown when mature, angular or sinuous, averaging 3–4 to a mm.; spores white, smooth, globose or subglobose, 2–3.5 μ in diameter.

On dead wood of deciduous trees, especially *Quercus*. June to September. Not common.

Distinguished by the uniform umber brown color of the whole plant, the soft and watery context, etc.

48. **P. gilvus** Schw. ex Fries, Elench. Fung. 1: 104. 1828.

Boletus gilvus Schw. Syn. Fung. Car. 96. 1822.

Plants annual or reviving for two or three years, sessile or effused-reflexed, often imbricate; pileus dimidiate, 1–7 x 2–12 x 0.2–2 cm., leathery or corky when fresh, woody and rigid when dry, yellowish brown or reddish brown, in very young stages covered by a purplish, villous pubescence, otherwise glabrous, usually rough, more or less zonate, margin thin and acute; context yellowish brown, soft-corky to woody, 0.1–1.3 cm. thick; tubes 1–5 mm. long, the mouths reddish brown or darker, circular, then angular, averaging 6–8 to a mm., the walls rather thick and entire; spores white, smooth, oblong-ellipsoid, 3–4 x 5–6 μ.

On dead wood of all kinds. July to December. Common.

Closely related to *P. radiatus* Sow. ex Fries and *P. cuticularis* Bull. ex Fries, but distinct in the white spores, the lighter colored surface and the more woody context. *P. isidiodes* Schw. as reported by Lea belongs here.

49. P. radiatus Sow. ex Fries, Syst. Myc. 1: 369. 1821.
Boletus radiatus Sow. Eng. Fungi *pl. 196*. 1799.

Plants annual, sessile or decurrent; pileus dimidiate or flabelliform and attached by an attenuate base, 2–5 x 2–7 x 0.3–2 cm., firm and rigid, yellowish brown or rust-colored, velvety to glabrous, sometimes conspicuously zonate, sometimes azonate, margin thin or thick, acute; context yellowish to rusty brown, corky and somewhat friable, 2–5 mm. thick; tubes 1–8 mm. long, the mouths grayish umber to rusty red, circular, then angular, averaging 4–5 to a mm.; spores (teste Bresadola) yellowish, elliptical, 3.5–4.5 x 5.5–6.5 μ.

Growing commonly on *Betula* and *Alnus*. Rare.

A species intermediate between *P. gilvus* Schw. ex Fries, and *P. cuticularis* Bull. ex Fries, distinguished from the former by the habitat, the brighter color and the smoother surface of the pileus, and by the colored spores, and from the latter chiefly in the habitat. The species was reported from Ohio by Lea but I have not examined the plants.

50. P. cuticularis Bull. ex Fries, Syst. Myc. 1: 363. 1821.
Boletus cuticularis Bull. Herb. Fr. *pl. 462*. 1809.

Plants annual, sessile, often imbricate; pileus dimidiate or flabelliform and attached by an attenuate base, 3–7 x 3.5–10 x 0.3–1 cm., spongy and fleshy-tough when fresh, leathery to rigid when dry, yellowish brown to rusty brown, compact wooly-tomentose, becoming fibrillose or almost glabrous, sometimes subzonate on the margin, margin thin, acute, often inflexed; context yellowish brown or rust-colored, tough and watery when fresh, distinctly fibrous, 2–7 mm. thick; tubes 2–7 mm. long, the mouths hoary brown to rust-colored, angular, averaging 3–5 to a mm.; spores yellowish brown, smooth, subglobose to broadly elliptical, 4.2–5.7 x 5.5–7 μ.

On dead wood of deciduous trees. August to November. Common.

This species is very closely related to *P. radiatus* Sow. ex Fries, but Ohio plants may be distinguished from that species by the habitat, the thicker and larger pileus, and by the more tomentose and spongy surface. *P. perplexus* Peck, the types of which have been destroyed, is thought by some to be this species and our plants are frequently referred to it.

51. P. hispidus Bull. ex Fries, Syst. Myc. 1: 362. 1821. *Boletus hispidus* Bull. Herb. Fr. *pl. 210.* 1791. *Polyporus endocrocinus* Berk. Hooker's Lond. Jour. Bot. **6**: 320. 1847.

Plants annual, sessile, sometimes imbricate; pileus dimidiate, 6–20 x 9–25 x 2–6 cm., spongy and watery when fresh, firm and rigid when dry, yellowish brown to rusty red, soft from the covering of the dense hirsute or hispid tomentum or pubescence, azonate, margin thick or thin, obtuse or acute; context usually light yellowish brown above and dark reddish brown next to the hymenium, fibrous, firm when dry, 1–5 cm. thick; tubes 0.6–1.5 cm. long, mouths yellowish brown becoming darker where bruised, circular, then angular, averaging 2–4 to a mm.; spores yellowish brown, smooth, broadly ovoid to ellipsoid, 6.5–7 x 7–9.5 μ.

On living trunks of deciduous trees. September to December. Rare.

Much larger than *P. cuticularis* Bull. ex Fries, and *P. radiatus* Sow. ex Fries, and expecially distinct by the hirsute or hispid pubescence. In point of size it more nearly approaches *P. dryadeus* Pers. ex Fries, and *P. dryophilus* Berk., but easily distinguished from them by the pubescence.

52. P. dryadeus Pers. ex Fries, Syst. Myc. 1: 374. 1821. *Boletus dryadeus* Pers. Obs. Myc. 3. 1799.

Plants sessile; pileus dimidiate, applanate, 6–30 x 8–35 x 2–6 cm., spongy and watery when fresh, more or less corky or woody when dry, grayish brown to dark brown or black in old specimens, glabrous, azonate, margin thick and obtuse, distilling drops of water when young and growing; context umber-brown to rust-colored, subshining when dry, soft and watery, corky or woody on drying, 1.5–4 cm. thick; tubes 0.3–2 cm. long, mouths grayish brown, darker on drying, circular, then angular, averaging 3–5 to a mm.; spores (teste Bresadola) globose or subangular, smooth, yellowish, 8–9 x 7–8 μ.

On living trunks of *Quercus*. September to November. Rare.

Very closely related to *P. dryophilus* Berk., and probably the two have been confused in this country. *P. dryadeus* is usually considered to be a more applanate form and much larger than *P. dryophilus*. There is also said to be a decided difference

in spore color in the two plants, *P. dryadeus* having much paler spores than *P. dryophilus*, but for this I cannot vouch. So far as known, *P. dryadeus* has not been collected in Ohio but the species has been reported from Michigan and Kentucky. Lloyd (Myc. Notes 36. *f. 383*) gives an illustration.

53. P. dryophilus Berk. Hooker's Lond. Jour. Bot. **6**:321. 1847.

Plants annual, sessile; pileus dimidiate, often ungulate, 3–12 x 7–20 x 1–10 cm., rather rigid, grayish brown, to reddish brown, scabrous with an innate, ferruginous pubescence, azonate or subzonate, margin thick and obtuse; context cinnamon or rusty brown, subshining, corky to hard and woody; tubes 0.3–2.5 cm. long, ferruginous-yellow within, the mouths cinnamon-brown, angular, averaging 2–3 to a mm.; spores ferruginous, smooth, ellipsoid to subglobose, 5 x 6.5 μ.

On living *Quercus* and on logs. August to November. Rare.

This species was originally described from specimens collected at Waynesville, Ohio, by Lea. To the description as given in Lea's catalogue the following note was added: "Nearly allied to *Polyporus dryadeus*, but a smaller, more rigid species with larger, differently colored pores. It has also much resemblance to *P. gilvus*."

54. P. Schweinitzii Fries, Syst. Myc. **1**:351. 1821.

Plants stipitate or sessile; pileus circular to dimidiate, 5–15 cm. broad, 0.5–1.5 cm. thick, spongy to soft-corky when fresh, firm, rigid, and sometimes friable when dry, ochraceous to orange-colored or brown in mature specimens, strigose-tomentose to almost glabrous, usually more or less zonate, margin thin or thick, acute; context yellowish to reddish brown, spongy when fresh, usually friable when dry, 0.2–1 cm. thick; tubes 1–6 mm. long, the mouths yellowish, darker when bruised and sometimes dark brown on drying, circular to angular and soon irregular, averaging 1–3 to a mm.; stipe present and well developed or entirely absent, central or excentric, agreeing in color, pubescence and consistency with the pileus, 0–6 cm. long, 1–2 cm. thick; spores (teste Lloyd) white, elliptical, smooth, 4 x 6 μ.

Growing on or about *Pinus*. Autumn. Rare.

This species is a very variable one, yet quite distinct in habi-

tat, consistency, pubescence, color, etc. It is known in Ohio only from a collection made at Cincinnati (now in the Lloyd Museum) by Mr. Wm. Holden. For illustrations see Lloyd, Myc. Notes, Polyp. Issue 1: *f. 208.*, aud von Schrenk, U. S. Dept. Agr., Div. Veg. Path. Bul. 25: *pl. 1. f. 1., pl. 2.*

55. P. circinatus Fries, Monogr. Hymen. Suec. **2**: 268. 1863.

Pileus stipitate or substipitate, circular to spathulate or flabelliform, convex to depressed, 3–9 cm. broad, 0.3–1 cm. thick, rather soft when fresh, firm when dry, yellowish to umber-brown, tomentose to velvety, azonate or subzonate, margin rather thin, acute; context yellowish to cinnamon-brown, duplex, soft and spongy above, firm next to the tubes, 1–6 mm. thick; tubes 1.5–4 mm. long, the mouths whitish to cinnamon, subcircular to angular, averaging 2–4 to a mm.; stipe sometimes rudimentary, usually lateral or excentric, fulvous to dark brown, tomentose, soft, up to 5 cm. long, 0.5–1.5 cm. thick; spores (teste Lloyd) pale color, 3 x 5 μ.

In coniferous and deciduous woods.

The species has not been reported from Ohio. It is distinguished by the duplex character of the context and by the poor development of a stipe. It is a question whether it is distinct from *P. tomentosus* Fr. Certainly *P. dualis* Peck is the same plant. Lloyd regards American plants in which the context is always duplex as belonging under *P. circinatus* Fries, and European plants with a uniform context as *P. tomentosus* Fries. The plant is illustrated by Lloyd (Myc. Notes Polyp. Issue *f. 198–99*).

56. P. obesus (Ellis & Ev.) Overholts, n. comb.

Polystictus obesus Ellis & Ev. Bull. Torr. Bot. Club **24**: 125. 1897.

Stipitate. Stipe central, spongy, velutinous, dark cinnamon, 4–6 cm. high, 0.5–1.5 cm. thick above, enlarged below to 1–3 cm.; pileus convex then depressed in the center, obconical at first with the margin obtuse, then spreading out with the margin acute, color lighter than that of the stipe, yellowish cinnamon, surface uneven, subcolliculose, not zonate, 4–6 cm. across; pores irregular, short (1 mm.), at first round with margins thick, finally irregular and subsinuous, 0.5–1 mm. across, margins acute; spores elliptical, ferruginous, 7-8 x 4–5 μ.

On the ground, in contact with and partly attached to decaying pine limbs partly buried in the soil. (The above description is according to Ellis and Everhardt, Bull. Torr. Bot. Club. **24**: 125. 1897.)

Distinguished from the next three species by the greater thickness of the pileus and stipe. From *P. circinatus* Fries, it is separated by the absence of a duplex context and by the slightly smaller pores. The plant is recorded by Morgan as *P. Montagnei* Fries, but according to Lloyd the record is based on plants collected in Canada by Dearness. It is listed in Lea's catalogue under the same name.

57. P. focicola Berk. & Curt. Jour. Linn. Soc. Bot. **10**: 305. 1868.

Pileus stipitate, circular in outline, convex-depressed to umbilicate, 2–4 cm. broad, 1–6 mm. thick, coriaceous when fresh, rigid when dry, grayish brown to cinnamon, finely tomentose, striate, zonate, margin thin and acute; context cinnamon-brown, fibrous, less than 0.5 mm. thick; tubes 1–6 mm. long, the mouths angular or irregular, cinnamon to rusty brown, averaging 1 mm. or more in diameter; stipe central, light to dark brown, minutely velvety, 1.5–3 cm. long, 2–4 mm. thick; spores (teste Lloyd) pale colored, smooth, elliptical, 5 x 10 μ.

On burned earth in woods. July to November. Rare.

The species differs from *P. perennis* L. ex Fries only in the much larger pores. The plants were reported by Lea as *P. connatus* Schw. and by Morgan as *P. parvulus* Klotzsch. The plant is well illustrated by Lloyd (Myc. Notes Polyp. Issue 1: *f. 203–4*).

58. P. perennis L. ex Fries, Syst. Myc. **1**: 350. 1821.

Boletus perennis L. Sp. Plant. 1177. 1753.

Pileus stipitate, circular in outline, convex-depressed to umbilicate, 1.5–7 cm. broad, 1–3 mm. thick, coriaceous, rigid when dry, grayish brown to cinnamon or rust-colored but never silky, finely tomentose, zonate, margin thin and acute; context cinnamon-brown, fibrous, less than 1 mm. thick; tubes 1–2.5 mm. long, the mouths grayish to cinnamon, angular, averaging 2–4 to a mm.; stipe central or subcentral, cylindrical, concolorous with the pileus, velvety, 1.5–5 cm. long, 1–6 mm. thick; spores (teste Lloyd) pale colored, 4–5 x 8–10 μ.

Growing on burned earth. July to November. Not common.

The plant closely resembles the next species but is separated from it by the habitat and the dull cinnamon or cinnamon-gray color of the zonate pileus. *Polystictus proliferus* Lloyd is said by its author to be a form of this species. It was collected near Cleveland. This species is illustrated by Atkinson (Mushrooms *f. 187*), Hard (Mushrooms *f. 346*), and Lloyd (Myc. Notes Polyp. Issue 1: *f. 201*).

59. P. cinnamomeus Jacq. ex. Fries, Epicr. Syst. Myc. 429. 1838.

Boletus cinnamomeus Jacq. Coll. Bot. etc. **1**: 116. 1786. *P. subsericeus* Peck, Ann. Rept. N. Y. State Mus. **33**: 37. 1880.

Pileus stipitate, circular in outline, convex-depressed to umbilicate, 1–5 cm. broad, 1–3 mm. thick, pliant and tough, bright cinnamon-rufous to bright amber-brown, silky fibrillose, the fibrils sometimes suberect towards the center of the pileus, silky striate, sometimes zonate, margin thin and acute; context cinnamon or rusty brown, fibrous, less than 0.5 mm. thick; tubes not more than 2 mm. long, the mouths rufous-cinnamon, angular, averaging 2–4 to a mm.; stipe central, cylindrical, concolorous with the pileus, velvety to villous, 1–4 cm. long, 1–3 mm. thick; spores (teste Lloyd) pale colored, elliptical, smooth, 5–6 x 7–10 μ.

Most frequently on clay banks, usually among moss. July to September. Not common.

Distinguished from *P. circinatus* Fries, and *P. obesus* Ellis & Ev. by the very thin context; from *P. perennis* L. ex Fries by the silky pileus and the habitat; from *P. focicola* Berk. & Curt. by the much smaller pores. For illustrations see Lloyd, Myc. Notes Polyp. Issue 1: *f. 200.*, and Bresadola, Fungi Trid. *pl. 99.*

60. P. lucidus Leyss. ex Fries, Syst. Myc. **1**: 353. 1821.

Boletus lucidus Leyss. Flora Halensis 300. 1783. [2nd ed.] *Ganoderma sessile* Murr. Bull. Torr. Bot. Club **29**: 604. 1902. *Ganoderma subperforatum* Atk. Bot. Gaz. **46**: 337. 1908.

Plants stipitate or sessile, annual; pileus dimidiate or reniform in outline, 3-12 x 3.5-20 x 0.4-2.5 cm., coriaceous-corky when fresh, corky or woody when dry, the upper surface covered by an encrusting persistent layer of deep reddish chestnut varn-

ish, often wrinkled, glabrous or pruinose from a coating of brown conidial (?) spores, zonate or concentrically sulcate, the margin thin and acute, sometimes lobed; context whitish to light brown, sometimes separated into an upper, light colored, soft layer, and a lower darker and firmer layer, but often uniform in color and texture, 0.2–1.5 cm. thick; tubes 0.3–1.5 cm. long, not decurrent, the mouths white or umber, darker when bruised, circular to angular, averaging 3–5 to a mm.; the hymenium often with red-varnished patches on which no tubes are produced; stipe often entirely absent, lateral when present, covered like the pileus, 1–10 cm. long, 0.5–1 cm. thick; spores yellowish brown, smooth or apparently slightly verrucose, ovoid with a truncate base, 5–6.3 x 9.4–11μ.

On stumps and trunks of dead or injured deciduous trees. Common.

The variation in the pileus from stipitate to sessile may be confusing at first, but the deep chestnut-red color, not changing to yellowish as in the next species, will usually be found to be the distinguishing character of the species. The plant is described by Murrill under the name of *Ganoderma sessile* Murr. Atkinson has described a new species of *Ganoderma* from Ohio under the name of *G. subperforatum*. At the writer's request Professor Atkinson very kindly sent the type collection for examination. Under the ordinary high power of the microscope the spores of both *P. lucidus* and *G. subperforatum* appear to be practically smooth. By the use of the oil-immersion lens varying degrees of apparent echinulation are to be made out in the ordinary forms of *P. lucidus* while in the type collection of *G. subperforatum* the spores do not have that appearance, although Professor Atkinson states that by first boiling the spores in potassium hydroxide solution the perforations in the spore walls are faintly visible. I am convinced, however, that the echinulate appearance when present is not due to projections on the outer wall, but, as Atkinson has said, to perforations in the inner spore wall. An examination of the dozen or more collections of *P. lucidus* in my own herbarium have given evidence of a great variability in this character. Since *G. subperforatum* is not otherwise to be distinguished from *P. lucidus*, it has seemed best to consider the name as a synonym in this paper. Even

were the character constant one might well question the advisability of separating the species on a character that requires the use of the oil-immersion lens for its detection.

This and the following species are included in the genus *Fomes* by Saccardo, and many writers have followed his example. Why this should be done is not clear, for both species are always annual and the tubes are never stratified. The following illustrations will aid in determination: Atkinson, Mushrooms *f. 188*; Bot. Gaz. **46**: *f. 5.*, and Hard, Mushrooms *f. 332*.

61. P. Curtisii Berk. Hooker's Jour. Bot. Kew Gard. Misc. 1: 101. 1849.

Pileus stipitate, reniform or flabelliform in outline, 3–12 x 3–20 x 0.7–2 cm., coriaceous-corky when fresh, corky when dry, covered with a thin chestnut or reddish varnish that soon begins to disappear, leaving the pileus yellowish or sometimes almost white, glabrous, zonate or concentrically sulcate, the margin rather thick, sometimes truncate; context in two layers, a yellowish or pallid upper layer, rather soft in texture, and a brownish lower layer next to the hymenium, firm or corky in texture, the whole 0.5–1 cm. thick; tubes 0.3–1.2 cm. long, not at all decurrent, the mouths white to brownish, mostly circular, averaging 3–5 to a mm.; stipe always lateral, cylindrical, persistently red-varnished and encrusted, sometimes bluish in color, the context in two layers as in the pileus, 2–10 cm. long, 0.5–3 cm. thick; spores brown, ovoid to elliptic, smooth or appearing minutely echinulate, with a heavy outer wall, 4.6–7.2 x 8.5–11.8 μ.

On and about stumps and trunks of trees. Rare.

This is typically a more southern plant and is rarely found north of the Ohio River. It is distinguished from the preceding species by the yellowish color assumed by the mature pileus, the change in color being due to the disappearance of the reddish varnish. It is sometimes classed as a *Fomes* but is probably never truly perennial. For illustration see Atkinson, Bot. Gaz. **46**: *f. 1–3.*

SPECIES DOUBTFUL OR EXCLUDED

The following species reported by either Morgan or Lea are now believed to have been misdetermined, but the writer does not know to what species the plants should be referred: *P. ovinus* Schaeff. ex Fries; *P. leucomelas* Pers. ex Fries; *P. lentus* Berk.; *P. fragilis* Fries; and *P. badius* Schw.

P. intybaceus Fries reported by Morgan is possibly a form of *P. giganteus*, *P. frondosus*, or a closely related species.

P. phœoxanthus Berk. was originally described from material collected in Ohio by Sullivant. The type specimen is said to be in fragments and the plant has never been collected since Sullivant's time.

FOMES Fries, ex Gill.

Champ. Fr. 682. 1878. Fries; Nov. Symb. 31. 1851.

Plants typically perennial, epixylous, sessile (in our species); pileus corky or more often woody in texture, often becoming rimose, anoderm, or encrusted; context white, reddish, or brownish, soft and punky to hard and woody; tubes as in *Polyporus* except that they are arranged in definite or indefinite layers corresponding to periods of growth of the plant, the mouths circular or angular, never dædaloid or irpiciform; spores white or brown.

The genus *Fomes* includes all of the perennial forms which have the tubes as in the genus *Polyporus*. Each season one layer of tubes is produced and plants of the first season's growth are likely to be referred to the genus *Polyporus*. The key to that genus has been made to include a few such forms, the descriptions of which are always to be sought in the genus *Fomes*. A few species are so constantly annual in duration that they might perhaps better be included in the genus *Polyporus*.

KEY TO THE SPECIES

Context white or only slightly colored (*Species with wood-colored, flesh-colored, or rose-colored context included here*) 1
Context yellowish brown or darker 7
1. Sporophore small, scarcely more than 2 cm. broad; context white 2
1. Sporophore larger, more than 2 cm. broad; context whitish or somewhat colored 3

2. Pileus entirely dark brown or black; plants growing only on the wood of *Alnus* and *Hamamelis* ..... *1. F. scutellatus*
2. Pileus not entirely black, the margin at least remaining white; plant growing on the wood of other deciduous trees, often on structural timber ..... *2. F. ohiensis*
3. Hymenium or context pinkish or reddish 4
3. Hymenium or context whitish or yellowish 5
4. Tubes more than 3 mm. long; plants usually growing on stumps and trunks of *Fraxinus* ..... *6. F. fraxineus*
4. Tubes not more than 3 mm. long; plants usually growing on the wood of coniferous trees ..... *7. F. carneus*
5. Hymenium distinctly stratified, the strata of tubes separated by distinct layers of context; mouths of the tubes angular, usually glistening .. *4. F. connatus*
5. Hymenium indistinctly stratified or if somewhat distinctly so the layers not separated by distinct layers of context; mouths of the tubes mostly circular, not glistening 6
6. Plant growing on dead wood, usually of coniferous trees; mouths of the tubes small, averaging 3–5 to a mm. ..... *5. F. pinicola*
6. Plant growing only on living *Fraxinus;* mouths of the tubes rather large, averaging 2 to a mm. ..... *3. F. fraxinophilus*
7. Pilei forming a densely imbricate, globose or cylindrical mass .... *8. F. graveolens*
7. Pilei not forming a densely imbricate, globose or cylindrical mass 8
8. Surface of the pileus not distinctly encrusted 9
8. Surface of the pileus distinctly encrusted 13
9. Context less than 5 mm. thick; sporophore often effused-reflexed or entirely resupinate; growing usually on dead wood ..... *9. F. conchatus*
9. Context more than 5 mm. thick; sporophore generally sessile; often growing on living trees 10
10. Sporophore found only on *Robinia* ..... *11. F. rimosus*
10. Sporophore found on some other host 11
11. Tubes in the older layers distinctly white encrusted or stuffed 12
11. Tubes in the older layers not distinctly white encrusted or stuffed *12. F. Everhartii*
12. Surface of the pileus black, somewhat shining, and rimose; margin rather thin and acute ..... *13. F. igniarius*
12. Surface of the pileus dull brown; margin thick and somewhat obtuse .... *14. F. nigricans*
13. Encrusting layer thin, easily indented; plants annual or sometimes reviving the second season but with the pileus distinct from and coming out below that of the first season ..... *17. F. lobatus*
13. Encrusting layer thick and horny; plants strictly perennial 14
14. Plant growing only on species of *Prunus* ..... *10. F. fulvus*
14. Plant growing on some other host 15
15. Context hard and woody ..... *13. F. igniarius*
15. Context punky 16
16. Mouths of the tubes medium-sized, averaging 3 to a mm.; spores white *15. F. fomentarius*
16. Mouths of the tubes minute, averaging 5 to a mm; spores brown ..... *16. F. applanatus*

1. **F. scutellatus** Schw. ex Cooke, Grevillea 14: 19. 1885.
Polyporus scutellatus Schw. Trans. Am. Phil. Soc. II. 4: 157. 1832.

Plants perennial, sessile, often attached by the apex of the pileus; pileus dimidiate or circular, convex, 0.5–1.5 x 0.5–2 x 0.1–0.5 cm., corky when fresh, hard and woody when dry, dark brown or black at least when mature, velvety, azonate or somewhat concentrically sulcate, margin rather thick, acute; context white to wood-colored, corky, not more than 2 mm. thick; tubes 1–2 mm. long, indistinctly stratified, the mouths white to umber, circular or subcircular, averaging 4–5 to a mm.

Chiefly on dead limbs of *Alnus* and *Hamamelis*. Rare.

This species is distinguished from *F. ohiensis* Berk. ex Murrill by the habitat and the black surface of the entire pileus including the margin. Specimens have been received from Mr. Claassen, Cleveland, Ohio.

2. **F. ohiensis** Berk. ex Murrill, Bull. Torr. Bot. Club 30: 230. 1903.
Trametes ohiensis Berk. Grevillea 1: 66. 1872.

Plants perennial, sessile, often attached by the vertex of the pileus; pileus dimidiate or shield-shaped, convex to ungulate, 0.5–3 x 0.5–4 x 0.2–1 cm., soft-corky when fresh, hard and woody when dry, at first pure white but becoming cinereous or yellowish and often black at the base but the margin remaining white, finely tomentose to glabrous, often zonate or concentrically sulcate, margin rather thick, acute or obtuse; context white to wood-colored, soft-corky to woody, 1–3 mm. thick; tubes 1–5 mm. long, often arranged in more or less definite rows, indistinctly stratified in two to six layers, the mouths white, circular, averaging 3–5 to a mm., the dissepiments almost as thick as the diameter of the pores.

On dead wood of deciduous trees, especially on structural timber. Common.

By its small size this species is separated from all perennial forms except *F. scutellatus* Schw. ex Cooke. It differs from that species in habitat and in the margin of the pileus always remaining white.

3. **F. fraxinophilus** Peck ex Sacc. Syll. Fung. 6: 172. 1888.
Polyporus fraxinophilus Peck, Ann. Rept. N. Y. State Mus. 35: 136. 1882.

Plants perennial, sessile or effused-reflexed, often imbricate; pileus dimidiate, convex to compressed-ungulate, 2–25 x 3.5–40 x 1.5–10 cm., woody, white at first, becoming blackish and often somewhat rimose with age, not encrusted, soon glabrous, concentrically sulcate, margin thick, obtuse or acute; context white to cinnamon wood-color, corky or woody, 0.5–1 cm. or more thick; tubes 2–3 mm. long, indistinctly stratified in many layers, the mouths white to cinereous or yellowish, circular, averaging 2–3 to a mm., the walls thick and entire; spores white, smooth, ellipsoid to ovoid or pyriform, 5–6.3 x 7.3–8 μ.

Growing only on living trunks of *Fraxinus*. Common.

In habitat the species corresponds closely to *F. fraxineus* Bull. ex Cooke, from which it differs in the entire absence of any rosy or reddish colors and in being always perennial. An excellent illustration is given by Hard (Mushrooms *f. 350*).

4. **F. connatus** Weinm. ex Gill. Champ. Fr. 1: 684. 1878.
Polyporus connatus Weinm. Fl. Ross. 332. 1836. *P. connatus* Fries, Epicr. Syst. Myc. 472. 1838.

Plants perennial, sessile or effused-reflexed, sometimes imbricate; pileus dimidiate, convex, 2–10 x 3–15 x 0.5–4 cm., corky when fresh, somewhat woody when dry, whitish, cinereous, or slightly yellowish, sometimes blackish toward the base, not encrusted, velvety-tomentose to glabrous, usually azonate, margin thick, acute or obtuse; context white or pallid, punky to soft corky, 0.3–1 cm. thick; tubes 1.5–5 mm. long, distinctly stratified, the different strata separated from each other by a thin layer of context, the mouths whitish to yellowish, glistening, angular, averaging 4–5 to a mm., the walls entire to slightly dentate; spores (teste Bresadola) white, globose, 3–4 μ in diameter.

Growing on living deciduous trees, more often at the bases of species of *Acer*, and frequently covered with moss. Common.

The distinguishing characters are the habitat, the layers of context interposed between successive layers of tubes, and the glistening mouths of the tubes. In but one other species of *Fomes* do we find the second character developed and that

is in *F. applanatus* Pers. ex Wallr. That species always grows on old logs and stumps and has a rusty brown context.

Bresadola and Murrill regard *F. populinus* Schum. ex Cooke, to be the same plant as this species. This may be the case but the figure of *F. connatus* in Fries' 'Icones' (*f. 185*) represents our plant much better than the figure of *F. populinus* in 'Flora Danica' (*pl. 1791*). Most of the specimens distributed in exsiccati in both this country and Europe are under the former name and that one is here given preference. A study of the types of both species should show whether they are the same or not, but from the evidence at hand our plants must be referred to *F. connatus*.

5. F. pinicola Sw. ex Cooke, Grevillea 14: 17. 1885.

Boletus pinicola Sw. Sv. Vet. Akad. Handl. 88. 1810. *Polporus pinicola* Fries, Syst. Myc. 1: 372. 1821.

Plants perennial, sessile; pileus plane to convex, rarely ungulate, dimidiate, 4–15 x 6–20 x 3–10 cm., woody and rigid, grayish to black, partly or entirely covered with a reddish gluten that forms a crust over the surface, glabrous, sometimes concentrically sulcate, margin thin or thick, often obtuse; context pallid or wood-colored, corky to woody, 0.5–2 cm. thick; tubes 3–5 mm. long, distinctly or indistinctly stratified, the mouths white to umber, circular, averaging 3–5 to a mm., the walls thick and entire.

On dead wood, usually of coniferous trees.

Distinguished from closely related species by the resinous, somewhat sticky, reddish crust found on the pileus. The plant is common wherever coniferous woods are found. Hard (Mushrooms *f. 348*) gives a photograph of it but does not state that he ever collected it in Ohio.

6. F. fraxineus Bull. ex Cooke, Grevillea 14: 21. 1885.

Boletus fraxineus Bull. Herb. Fr. *pl. 433. f. 2.* 1789. *Polyporus fraxineus* Bull. ex Fries, Syst. Myc. 1: 374. 1821.

Plants annual or perennial, sessile, sometimes imbricate; pileus dimidiate, 4–10 x 6–15 x 0.6–2 cm., corky when fresh, rigid and woody when dry, light colored, always with reddish or reddish brown stains, or altogether reddish, encrusted with a thin hard crust, minutely velvety to glabrous, more or less zonate, margin thin or thick, acute; context floccose-punky

to corky, whitish or pallid when dry, tinged pinkish or flesh-colored when fresh, 0.2–2 cm. thick; tubes 2–6 mm. long, usually in a single layer but sometimes stratified, mouths whitish, pallid, or flesh-colored, circular or subcircular, averaging 4–6 to a mm., the dissepiments rather thick and entire; spores (teste Murrill) subglobose, smooth, subhyaline, 5–6 x 6–7 μ.

Usually found on living *Fraxinus* but sometimes on other hosts. Rare.

The habitat, the reddish blotches on the pileus, and the pinkish hymenium and context in fresh specimens will identify the plant. But three collections are known from Ohio; one by Morgan, one by W. G. Stover near Columbus, in 1910, and one near Camden, Ohio, by the writer, in 1912. All of these collections are of the annual form.

7. F. carneus Nees ex Cooke, Grevillea **14**: 21. 1885.

Polyporus carneus Nees, Nova Acta Acad. Leop. Carol. **13**: *pl. 3.* 1827.

Plants annual or perennial, sessile; pileus dimidiate, 1.5–5 x 1.5–10 x 0.3–1.5 cm., soft-corky when fresh, firmer when dry, pinkish or rosy, sometimes blackish with age, velvety to glabrous, usually azonate, margin thin and acute; context pinkish or rosy, floccose or punky to soft-corky, 0.2–1 cm. thick; tubes 0.5–3 mm. long, usually in a single layer but sometimes stratified, mouths pinkish or rosy, circular or subcircular, averaging 3–5 to a mm., dissepiments thick and entire.

Usually on wood of coniferous trees. Rare.

The species will be recognized by the uniform pinkish color of the whole plant. The color is well retained on drying. Specimens are in the herbarium of the New York Botanical Garden, collected by James, in Ohio. Authorities disagree as to the identity of *F. carneus* and *F. roseus* Fries ex Cooke.

8. F. graveolens Schw. ex Cooke, Grevillea **13**: 118. 1884.

Boletus graveolens Schw. Syn. Fung. Car. 97. 1822. *Polyporus conglobatus* Berk. Hooker's Lond. Jour. Bot. **4**: 303. 1845.

Plant composed of numerous overlapping pilei arising from a central solid core and forming a more or less globose or cylindrical mass 5–12 cm. in diameter; pilei not more than 2 cm. broad, but connate laterally, corky when fresh, rigid and firm when dry, grayish brown to dull cinnamon-brown, becoming

black in weathered specimens, slightly encrusted, pulverulent to glabrous, azonate or marked with fine grayish zones, margin thick, deflexed and almost concealing the pores; context fulvous to golden brown rust-colored, floccose-fibrous, 1-4 mm. thick; tubes 2–4 mm. long, the mouths grayish, hoary brown, or umber, circular, averaging 3–4 to a mm., the dissepiments thick and entire.

On logs or trunks of deciduous trees, especially *Fagus*, *Quercus*, and *Acer*. Rare.

A characteristic plant that will be recognized at sight. It is commonly known as "sweet knot" from the sweet, powerful odor that it is said to give off. The writer has made four different collections of this rare plant in different stages of growth but has never been able to detect the slightest semblance of a sweet odor. The plant is exceptionally well illustrated by Lloyd (Myc. Notes, Polyp. Issue 3: *f. 367–68;* Syn. Stip. Polyp. *f. 455*), and Hard (Mushrooms *f. 334*). The first and the last of the figures cited are upside down.

9. F. conchatus Pers. ex Gill. Champ. Fr. 1:685. 1878. *Boletus conchatus* Pers. Obs. Myc. 1:24. 1796. *Polyporus conchatus* Fries, Syst. Myc. 1:376. 1821.

Plants perennial, sessile, or more often effused-reflexed and frequently entirely resupinate; pileus dimidiate to conchate, 0–7 x 4–12 x 0.2–3.5 cm., woody, grayish brown, yellowish brown or black, rarely encrusted, tomentose at least on the margin, becoming glabrous behind, zonate or concentrically sulcate and sometimes somewhat rimose, margin thin, mostly acute; context yellowish brown to dark brown, woody, 1.5–3 mm. thick; tubes 1–2 mm. long, indistinctly stratified, mouths fulvous to dark brown, usually glistening, circular or subcircular, averaging 4–6 to a mm.

On dead wood, rarely on living trees. Common.

The plant is most frequently found entirely resupinate on old oak logs. Distinctly sessile forms are sometimes found, especially on living trees. The hymenium usually has a silky luster when viewed in changing positions, and on the whole the plant is so characteristic that when once recognized, the collector usually has no trouble with subsequent collections notwithstanding the fact that the species often presents great

differences in size and habit. In Europe the plant is usually known as *F. salicinus* Pers. ex Gill. and it was so reported from Ohio by Morgan. It is entirely different from all other species of *Fomes* except *F. fulvus* Scop. ex Gill. and *F. ribis* Schum. ex Fries in the thin pileus, often conchate in form and with a concave hymenium. Usually the pileus is not more than 1 cm. thick. *F. fulvus* Scop. ex Gill. is distinct in its habitat as is also *F. ribis* Schum. ex Fries.

10. **F. fulvus** Scop. ex Gill. Champ. Fr. 1: 687. 1878.

Boletus fulvus Scop. Fl. Carn. 2:469. 1772. [2nd ed.] *Polyporus fulvus* Fries, Epicr. Syst. Myc. 466. 1838.

Plants perennial, sessile, effused-reflexed or entirely resupinate; pileus dimidiate, convex, 0–4 x 3–8 x 0.5–3 cm., woody, fulvous to ferruginous when young, becoming grayish black or black in age, encrusted, minutely velvety to glabrous, sometimes sulcate, margin rather thick, acute or obtuse; context dark brown, woody, 3–8 mm. thick; tubes 2–4 mm. long, rather distinctly stratified, the mouths circular to slightly angular, grayish brown to tawny, averaging 4–5 to a mm., dissepiments rather thick, entire.

Growing only on wood of species of *Prunus*. Not common.

One should have no trouble in identifying this species if the habitat is taken into consideration as it is the only perennial form that grows on *Prunus*. Morgan reported it under the name of *F. supinus* Schw.

11. **F. rimosus** Berk. ex Cooke, Grevillea 14:18. 1885.

Polyporus rimosus Berk. Hooker's Lond. Jour. Bot. 4:54. 1845. *Pyropolyporus robiniae* Murrill, Bull. Torr. Bot. Club 30:114. 1903.

Plants perennial, sessile; pileus dimidiate, convex to ungulate, 3–20 x 6–30 x 1.5–10 cm., woody, at first fulvous, becoming dark brown or black, not encrusted, velvety in young specimens, glabrous and very rimose in old plants, concentrically sulcate, margin thick or thin, obtuse or acute; context fulvous to rusty brown, woody, 0.5–3 cm. thick, zonate; tubes 1–5 mm. long, indistinctly stratified in many layers, the mouths fulvous to rusty brown, circular, averaging 5–6 to a mm., walls rather thick and entire; spores brown, smooth, globose, 4–5μ in diameter.

Growing only on living trunks of *Robinia*. Common.

The type locality for *F. rimosus* is given by Berkeley as the Swan River, Australia, and not Demerara and the Cape of Good Hope, as cited by Saccardo and by Murrill. If the specimens Murrill examined are from the two latter places, it is still possible that our plants belong under *F. rimosus*. Our species also occurs in South Africa as specimens examined from that locality agree well with our plants.

The plant is never found on any other host than the locust tree. This will distinguish it from all of its allies. Its closest relatives appear to be *F. Everhartii* Ellis & Gall. and *F. igniarius* L. ex Gill. The plant is well illustrated by Hard (Mushrooms *f. 347*), and by von Schrenk (Ann. Rept. Mo. Bot. Gard. **12**: *pl. 2*).

12. F. Everhartii Ellis & Gall.[1]

Mucronoporus Everhartii Ellis & Gall. Jour. Myc. **5**:141. 1889.

Plants perennial, sessile or decurrent; pileus dimidiate, convex, rarely ungulate, 2.5–10 x 4–20 x 2–6 cm., woody, entirely fulvous when young but becoming grayish brown or black and rough and rimose with age, velvety when young, glabrous when mature, scarcely encrusted, concentrically sulcate with age, margin thin or thick, acute or obtuse, usually remaining fulvous in color; context fulvous to rusty brown, shining (at least in herbarium specimens), zonate, woody, 1–4 cm. thick; tubes 3–6 mm. long, indistinctly stratified, tubes of the older layers sometimes partly stuffed with mycelium, the mouths concolorous with the context, circular, averaging 4–5 to a mm., the walls rather thin but entire, sometimes glistening; spores distinctly brown, smooth, globose, 4–5.3 μ in diameter.

On living trees, usually of *Quercus*. Not uncommon.

Distinguished from *F. igniarius* L. ex Gill. and *F. nigricans* Fries ex Gill. by the absence of the distinct encrustation or stuffing of the tubes in the old layers, by the more shining context, the somewhat thinner dissepiments, the hyaline spores, and the absence of a distinct crust on the pileus. The two

[1] *F. Everhartii* was originally described under the genus *Mucronoporus* and as far as I have been able to find, no specific statement of transfer to the genus *Fomes* was ever made. At the present time I have not been able to satisfy myself as to who was the first to make (unknowingly, it seems) the new combination, and therefore I do not know to whom credit for the transfer should be given.

species are closely related, however, and without the spores it is sometimes difficult to decide between them. The species differs from *F. fomentarius* L. ex Gill. and *F. applanatus* Pers. ex Wallr. in the unencrusted pileus, the woody context, and the short tubes.

13. **F. igniarius** L. ex Gill. Champ. Fr. 1:687. 1878.

Boletus igniarius L. Sp. Plant. 1176. 1753. *Polyporus igniarius* Fries, Syst. Myc. 1:375. 1821.

Plants perennial, sessile; pileus dimidiate, convex to somewhat ungulate, 2.5–11 x 4–25 x 1.5–12 cm., woody, grayish black, or entirely black, encrusted, sometimes somewhat rimose in age, glabrous, concentrically sulcate in older plants, margin rather thin, acute, usually grayish in growing specimens; context rusty red or rusty brown, scarcely shining, zonate, woody, 0.5–4 cm. thick; tubes 2–5 mm. long, usually indistinctly stratified, the older layers becoming distinctly whitish encrusted, the mouths circular, grayish brown to dark brown, averaging 4–5 to a mm., the walls thick and entire; spores (teste Romell) hyaline, subglobose, 5–7.5 x 4–7 μ, often 1-guttate.

On trunks of living deciduous trees. Not common.

In no other species is the stuffing or encrusting of the tubes by a whitish substance so evident as in this and the next one. In *F. Everhartii* Ellis & Gall. the tubes appear to be sometimes filled with a whitish mycelium but the character is scarcely evident except on close examination, while in *F. igniarius* and *F. nigricans* Fries ex Gill. in sections through the hymenium the whitish encrustation is plainly visible, and seems to be a distinguishing character. The plant is further to be distinguished from *F. Everhartii* by the hyaline spores, and the thicker dissepiments. The pores are somewhat smaller, but in measuring them the thick dissepiments are included, so that the number per mm. is about the same in the two species. From *F. fomentarius* L. ex Gill. and *F. applanatus* Pers. ex Wallr. the species is separated by the more woody context, the thinner crust, and the much shorter tubes, as well as by the hyaline spores. In *F. igniarius* the pileus is darker in color and is usually much more rimose than in *F. nigricans*. For illustrations see Atkinson, Cornell Univ. Agr. Exp. Sta. Bul. 193: *f. 78–4*.

14. F. nigricans Fries ex Gill. Champ. Fr., Hymen. 1: 685. 1878.

Polyporus nigricans Fries, Syst. Myc. 1:375. 1821.

Plants perennial, sessile; pileus dimidiate, convex to ungulate, distinctly triangular in cross-section, 5–10 x 7–13 x 2–7 cm., woody, dull brown or becoming brownish black, not encrusted, smooth or cracking somewhat in age but scarcely rimose, azonate or with one or two concentric furrows, the margin thick, acute or obtuse, with a broad ferruginous band; context rusty brown, zonate, woody, 0.6–2 cm. thick; tubes 2–7 mm. long, distinctly or indistinctly stratified, becoming distinctly white encrusted or stuffed in the older layers, the mouths dark brown, circular, minute, averaging about 5 to a mm., the walls thick and entire; spores white, subglobose or globose, 6.5 μ in diameter.

On trunks of trees, especially on *Betula*. Not common.

I have one collection of this fungus from W. A. Kellerman. The species has been confused with the preceding one from which it differs in the smoother and differently colored pileus and in being more decidedly triangular in cross-section. The best illustration is that given by Boudier (Ic. Myc. 1: *pl. 155*).

15 F. fomentarius L. ex Gill. Champ. Fr. 1:686. 1878.

Boletus fomentarius L. Sp. Plant. 1176. 1753. *Polyporus fomentarius* Fries, Syst. Myc. 1:374. 1821.

Plants perennial, sessile; pileus dimidiate, convex to strongly ungulate, 3.5–15 x 6–20 x 2–9 cm., hard and woody, grayish to cinereous, brownish, or black, covered with a thick horny crust that appears black and shining when cut, glabrous, smooth, never rimose, zonate or concentrically sulcate, margin thick and obtuse; context fulvous to ferruginous, never shining, punky to soft-corky, zonate, 0.3–3 cm. thick; tubes 0.5–2.5 cm. long, rather distinctly stratified, mouths grayish to cinnamon, averaging 3 to a mm., the walls thick and entire.

On living deciduous trees. Not common.

Distinguished from all of the preceding species by the punky or soft-corky context and the usually longer tubes. Most closely related to *F. applanatus* Pers. ex Wallr. but distinguished from it by the much longer pores and the hyaline spores. For

illustrations see Kellerman, Journ. Myc. **9**: *pl. 3.*, and White, Hymen. Conn. *pl. 35. f. 2.*

16. F. applanatus Pers. ex Wallr. Crypt. Fl. Ger. **2**: 591. 1833.

Boletus applanatus Pers. Obs. Myc. **2**: 2. 1799. *Polyporus applanatus* Fries, Epicr. Syst. Myc. 465. 1838. *P. leucophæus* Mont. Syll. Crypt. 157. 1856.

Plants perennial, sessile; pileus dimidiate, convex or plane, not ungulate, 3–30 x 5–50 x 1.5–7 cm., woody, usually grayish becoming brownish or blackish, glabrous, covered with a thick horny crust, zonate or concentrically sulcate, margin thin or thick, acute or obtuse; context dark ferruginous brown, floccose to soft corky, 0.6–2 cm. or more thick; tubes 0.5–1.5 cm. long, distinctly stratified after the first season, the strata separated by thin layers of context, mouths whitish to umber, darker when bruised, circular, minute, averaging 5–6 to a mm.

On dead wood of deciduous trees or on living trees. Common.

This is our most common species of *Fomes* and may be found in every woodlot, usually on stumps or old logs. It is distinguished from *F. fomentarius* L. ex Gill. by the more applanate pileus and the minute mouths of the tubes. For illustrations see Atkinson, Mushrooms *f. 15.*, White, Hymen. Conn. *pl. 35. f. 1.*, and Atkinson, Cornell Univ. Agr. Exp. Sta. Bul. 193: *f. 82.*

17. F. lobatus Schw. ex Cooke, Grevillea **14**:18. 1885.

Polyporus lobatus Schw. Trans. Am. Phil. Soc. II. **4**:157. 1832. *P. reniformis* Morgan, Journ. Cin. Soc. Nat. Hist. **8**:105. 1885.

Plants annual, frequently reviving for two or three years but the second year's growth distinct from and coming out below that of the first year, sessile or more often appearing substipitate; pileus dimidiate or reniform, plane, depressed, or somewhat convex, never ungulate, 4–12 x 4–15 x 1–3 cm., corky or somewhat flexible when growing, usually umber to yellowish or dark rusty brown, glabrous, covered with a thin, easily indented crust, zonate or concentrically sulcate, margin thin and acute; context dark rusty brown, soft and floccose to punky, 0.3–1 cm. thick; tubes 0.4–1 cm. long, not stratified, mouths circular or subcircular, white, yellowish or umber-

brown, darker when bruised, averaging 4 to a mm., walls rather thin but entire.

On dead wood of deciduous trees. Common.

This species is easily separated from *F. applanatus* Pers. ex Wallr. in that it is not perennial, and in that, if the plant revives the second year, the pileus comes out below that of the first year, and the latter persists as a dead decaying pileus. The second difference is in the character of the encrusting layer of the pileus. In *F. applanatus* Pers. ex Wallr. the crust is hard and horny and one cannot indent it with the thumb nail, while in *F. lobatus* the crust is thin, and often becomes cracked and brittle when old, but is always rather soft and easily indented.

TRAMETES Fries, Gen. Hymen. 11. 1836.

Plants annual or perennial, epixylous, sessile; pileus corky or woody in texture, small or medium sized; context white or brown (never red), descending into and forming the walls of the tubes; tubes typically appearing sunken into the context to unequal depths so that their bases are not in a continuous straight line; mouths circular or angular, never breaking up into teeth and rarely showing a daedaloid tendency.

One species here included in the genus is perennial, all the others are annual. The chief generic distinctions are the unequal depths to which the tubes are immersed in the context, and the homogeneous texture of the context and trama. The first distinction is often not apparent except on very close examination, and at times appears to break down entirely. Consequently, students will meet with some difficulty at times in deciding between the two genera, *Trametes* and *Polyporus*.

KEY TO THE SPECIES

Context white or whitish 1
Context brown or brownish 6
1. Pileus densely hirsute or hispid ..... *7. T. Peckii*
1. Pileus slightly pubescent to glabrous 2
2. Mouths of the tubes minute, averaging 4–6 to a mm. ..... *T. robiniophila*[1]
2. Mouths of the tubes larger, averaging 1–3 to a mm. 3
3. Pileus rather large; context more than 5 mm. thick; plant growing only on *Salix* ..... *3. T. suaveolens*
3. Pileus small, sometimes mostly resupinate; context less than 5 mm. thick; found on some other host 4

[1] For description of this plant see p. 104 under the genus *Polyporus*.

4. Hymenium light brown in color ..... *4. T. malicola*
4. Hymenium white or whitish 5
5. Pileus white or light colored; mouths of the tubes averaging 1–2 to a mm. *1. T. sepium*
5. Pileus cinnamon-brown; mouths of the tubes averaging about 3 to a mm. *2. T. serialis*
6. Sporophore woody, perennial; hymenium bright yellowish brown in color; mouths of the tubes often somewhat daedaloid; growing only on *Pinus* ..... *8. T. Pini*
6. Sporophore coriaceous or corky; hymenium white or dull brown; growing on wood of deciduous trees 7
7. Pileus hirsute or hispid 8
7. Pileus finely tomentose or glabrous 9
8. Mouths of the tubes large, averaging 1 to a mm.; pileus more than 4 mm. thick ..... *7. T. Peckii*
8. Mouths of the tubes medium sized, averaging 2–3 to a mm.; pileus less than 4 mm. thick ..... *6. T. rigida*
9. Context less than 1 mm. thick ..... *5. T. mollis*
9. Context more than 1 mm. thick ..... *4. T. malicola*

1. **T. sepium** Berk. Hooker's Lond. Jour. Bot. 6:322. 1847.

Plants annual, sessile or semirespuinate, imbricate or single; pileus dimidiate, 0.7–1 x 0.8–2.5 x 0.2–0.7 cm., flexible when fresh, corky when dry, grayish to pallid or wood-colored, minutely tomentose to glabrous, azonate, margin thin and acute; context white or pallid, tough when fresh, soft-corky when dry, less than 1 mm. thick; tubes 2–5 mm. long, mouths white or pallid, circular or rarely angular or sinuous, large, averaging almost 1 to a mm., the dissepiments thick and always entire; spores (teste Murrill) oblong, smooth, hyaline, 12 x 5 μ.

On fence posts, pickets, and other structural timber or dead wood.

Distinguished from *T. serialis* Fries by the short tubes, the whitish color of the pileus, and by the much larger mouths of the tubes; from *T. rigida* Berk. & Mont. by the lighter colored context, and the larger tube mouths.

2. **T. serialis** Fries, Hymen. Eur. 585. 1874. [2nd ed.]
Polyporus serialis Fries, Syst. Myc. 1:370. 1821.

Plants annual, sessile, effused-reflexed, or resupinate; pileus dimidiate, 0–1 x 1–4 x 0.3–0.8 cm., corky when fresh, hard and firm when dry, cinnamon-brown to coffee brown, glabrous, zonate, margin rather thick but acute; context white, fibrous, not more than 1 mm. thick; tubes 2–6 mm. long, the mouths

white or slightly discolored, sometimes slightly glistening, circular to angular, averaging 3 to a mm., the walls firm and entire; spores (teste Bresadola) hyaline, elongate, 7–10 x 3–3.5 μ.

On dead wood. Rare.

The white pores and the internally white tubes contrast strongly with the rich brown color of the pileus. It is distinct from *T. rigida* Berk. & Mont. in the glabrous, thicker pileus. From *T. sepium* Berk. it differs in the much smaller pores and the brown pileus; *T. malicola* has no white color in the tubes and the dissepiments are much thicker.

3. T. suaveolens L. ex Fries, Syst. Myc. 1:366. 1821.

Boletus suaveolens L. Sp. Plant. 1177. 1753.

Plants annual, sessile; pileus dimidiate, 3–9 x 6–14 x 1–3 cm., corky when fresh, firm and rigid when dry, white to grayish or slightly yellowish, finely villous-tomentose to glabrous, azonate, margin thin or thick, acute; context white or pallid, compact-corky to somewhat indurate, 0.5–2 cm. thick; tubes 0.2–1.5 cm. long, the mouths white or cinereous, circular to slightly angular, averaging 1–3 to a mm.

On dead or diseased *Salix*. Rare.

Distinguished from *T. Peckii* Kalchbr. by the prevailing whitish color and the more nearly glabrous pileus.

4. T. malicola Berk. & Curt. Journ. Acad. Nat. Sci. Phil. II. 3:209. 1856.

Plants annual or reviving for two or three seasons, effused-reflexed or entirely resupinate; pileus very narrow, 0–1 x 1–5 x 0.3–0.8 cm., coriaceous and leathery when fresh, corky when dry, avellaneous to cinnamon-brown or wood-colored, azonate, margin thick but acute; context wood-brown or lighter, soft-corky, 2–5 mm. thick; with a distinct pleasant odor when fresh; tubes 2–5 mm. long, sometimes indistinctly stratified in two or three layers, mouths wood-colored to cinnamon-brown, circular to angular or somewhat sinuous, averaging about 2 to a mm., the walls thick and entire; spores white, smooth, oblong, 2.8 –3.5 x 7.5–10 μ.

Growing on dead wood of deciduous trees, especially species of *Acer*. Common.

Entirely distinct from *T. sepium* Berk. in the semiresupinate

habit of growth, the prevailing dull brown color of both hymenium and pileus, and the smaller-mouthed tubes. In this last respect the plant more nearly approaches *T. serialis* Fries and *T. rigida* Berk. & Mont. From the former it is separated by the browner color of the hymenium, the lighter color of the pileus, the internally brown tubes, and the slightly larger and more irregular mouths. From the latter it differs chiefly in the more glabrous and less developed pileus and the longer tubes.

The type specimens of *T. malicola* were collected on the trunk of an apple tree by Schweinitz and referred by him to *P. populinus* Fries. Murrill has placed the name as a doubtful synonym for *P. galactinus* Berk. The writer has not examined the type of *T. malicola*, but our plants bear no resemblance to either *P. populinus* Fries or *P. galactinus* Berk. Our plants were determined by Lloyd and by Bresadola.

5. T. mollis Sommerf. ex Fries, Hymen. Eur. 585. 1874.

Daedalea mollis Sommerf. Suppl. Fl. Lapp. 271. 1826.

Plants annual or rarely reviving, rarely sessile, more often effused-reflexed or entirely resupinate; pileus dimidiate or elongate, 0–2.5 x 1–4 x 0.1–0.5 cm., coriaceous to rigid, umber-brown to almost black, finely tomentose to glabrous, zonate or multizonate, margin thin and acute; context light brown, fibrous, less than 1 mm. thick; tubes 2–3 mm. long, rarely in two or three layers, mouths light brown or grayish, subcircular to somewhat angular, often becoming sinuous or labyrinthiform, averaging 1–3 to a mm.; spores (teste Bresadola) elongate-ellipsoid, smooth, hyaline, 9–11 x 4–4.5 μ.

On dead wood. Rare.

The species differs from *T. rigida* Berk. & Mont. in the distinctly brown and almost glabrous pileus. From *T. serialis* Fries it differs in the light brown context, the much thinner pileus and the usually larger and more irregular pores. The context is much thinner than in *T. malicola* Berk. & Curt. and the general color is decidedly different.

6. T. rigida Berk. & Mont. Ann. Sci. Nat. III. **11**: 240. 1849.

Plants annual or rarely reviving, sessile, effused-reflexed or entirely resupinate, sometimes imbricate; pileus dimidiate,

0–3 x 2–6 x 0.1–0.3 cm., coriaceous when fresh, coriaceous or rigid when dry, cinereous to yellowish or slightly brownish, hirsute to hispid, usually zonate, sometimes with multicolored zones, margin very thin and acute; context light umber, fibrous, 0.5–3 mm. thick; tubes not more than 1 mm. long, the mouths white or brownish, circular to somewhat angular, averaging 2–3 to a mm., the walls rather thin but entire.

On dead wood. Not common.

Distinguished from all of its allies in the hirsute or hispid pubescence of the pileus. The pileus is thin and coriaceous and more nearly resembles the thin coriaceous species in *Polyporus*.

7. T. Peckii Kalchbr. Bot. Gaz. 6: 274. 1881.

Plants annual, sessile or effused-reflexed; pileus dimidiate, 1.5–6 x 2.5–12 x 0.5–2 cm., somewhat coriaceous when fresh, firm and rigid when dry, yellowish brown or reddish brown, densely hirsute or hispid, concentrically sulcate at times, margin thick or thin, acute; context light brown, fibrous, soft and spongy to firm and woody, 1–10 mm. thick; tubes 2–10 mm. long, the mouths dull brown or grayish brown, angular to irregular, averaging about 1 to a mm.; spores (teste Murrill) oblong or slightly curved, smooth, hyaline, 11–13 x 3.5–4 μ.

On dead wood of *Populus*, *Liriodendron*, and *Salix*. September to December. Frequent.

Easily recognized by the densely hirsute or hispid pubescence, the large pores, and the habitat. In Europe the species is known as *T. hispida* Fries.

8. T. Pini Thore ex Fries, Epicr. Syst. Myc. 489. 1838.

Boletus Pini Thore, Essai Chlor. Dep. Land. 487. 1803.

Plants perennial, sessile or effused-reflexed; pileus dimidiate, often ungulate, 3–15 x 5–20 x 1–6 cm., woody, yellowish brown to reddish brown or becoming black, the growing margin hirsute to tomentose, glabrous behind, zonate or concentrically sulcate, margin usually thick and somewhat obtuse; context yellowish brown to rusty brown, corky to woody, not more than 5 mm. thick; tubes 2–7 mm. long, indistinctly stratified, the mouths usually golden brown, subcircular to daedaloid and labyrinthiform; spores (teste Bresadola) hyaline, subglobose, 5–6 x 4–5 μ.

On coniferous wood. Rare.

The bright color of the hymenium usually contrasts strongly with the darker colors of the upper surface. *P. piceinus* Peck (=*Trametes Abietis* Karst.), which by some is regarded as a form of *T. Pini*, has never, to the writer's knowledge, been collected within the state.

SPECIES DOUBTFUL OR EXCLUDED

T. nivosus Berk. was erroneously reported from Ohio by Morgan. It is a tropical and subtropical species.

DAEDALEA Pers. ex. Fries,

Syst. Myc. 1: 331. 1821; Pers. Syn. Fung. 499. 1801.

Plants annual or rarely reviving for two or three years, sessile or effused-reflexed, growing on wood; pileus coriaceous to corky in texture, not encrusted; context white or whitish, fibrous or corky; hymenium typically daedaloid or labyrinthiform, but sometimes poroid, irpiciform or lamellate; spores white.

KEY TO THE SPECIES

Pileus small, thin and coriaceous, hirsute or villous; hymenium at first sinuous and dædaloid but soon breaking up into teeth.....*1. D. unicolor*

Pileus rather large and thick, corky, minutely velvety or glabrous; hymenium poroid, dædaloid, or somewhat lamellate but never breaking up into teeth..... 2

2. Mouths of the tubes less than 1 mm. broad.....*2. D. ambigua*

2. Mouths of the tubes more than 1 mm. broad..... 3

3. Pileus less than 1.5 cm. thick; walls of the tubes thin; plant found abundantly on *Salix*.....*3. D. confragosa*

3. Pileus more than 1.5 cm. thick; walls of the tubes thick; plant growing on *Quercus* and *Castanea*.....*4. D. quercina*

1. D. unicolor Bull. ex Fries, Syst. Myc. 1: 336. 1821.

Boletus unicolor Bull. Herb. Fr. *pl. 408*. 1788.

Plants annual or sometimes the marginal hyphæ reviving and continuing growth the second year, sessile, or effused-reflexed, imbricate; pileus dimidiate to flabelliform, 0.5–5 x 2–8 x 0.2–0.5 cm., coriaceous, white to cinereous or light brown, sometimes green from a covering of algæ, villous or hirsute, zonate or concentrically furrowed, margin thin, acute, sterile below; context white or pallid, fibrous, less than 1 mm. thick; tubes 1–4 mm. long, the mouths white to cinereous or umber, at first

dædaloid and sinuous, but soon breaking up into teeth—though retaining the sinuous character at the margin of the pileus—, averaging about 2 to a mm.

On dead wood. Common.

This plant may at first prove puzzling to the collector, as it was to me when first collected, for the thin, flexible pileus and the usually toothed hymenium indicate a close relationship with the thin coriaceous species of *Polyporus*, or even with *Irpex*. But the pores are decidedly sinuous, at least in young plants. The thin pileus and the hirsute or villous pubescence separate the species from other members of the genus.

2. D. ambigua Berk. Lond. Jour. Bot. **4**: 305. 1845.

Trametes lactea Berk. Hooker's Lond. Jour. Bot. 4: 305. 1845.

Plants annual or rarely reviving for two or three years, sessile, sometimes appearing substipitate; pileus dimidiate to reniform, 3–14 x 5–20 x 0.3–1.5 cm., slightly flexible when fresh, corky when dry, pure white to umbrinous, sometimes purplish black at the base, minutely velvety to glabrous, azonate or subzonate on the margin, margin rather thin, acute; context white or pallid, floccose-punky to corky, 0.2–1 cm. thick; tubes 2–4 mm. long, sometimes stratified in two or three layers, mouths whitish or yellowish, circular to sinuous and dædaloid, never lamellate, averaging 2–3 to a mm. in transverse direction, walls rather thick and entire.

On stumps and trunks of deciduous trees. Common.

Distinguished from *D. confragosa* Bolt. ex Fries by the white color, the white context, the smaller pores and the habitat. Hard (Mushrooms *f. 355–56*) gives excellent illustrations of the plant.

3. D. confragosa Bolt. ex Fries, Syst. Myc. **1**: 336. 1821.

Boletus confragosus Bolt. Hist. Fung. Suppl. **3**: 160. 1791.
Lenzites Cratægi Berk. Hooker's Lond. Jour. Bot. **6**: 323. 1847.

Plants annual, sessile; pileus dimidiate, 2–10 x 3–15 x 0.2–1.5 cm., slightly flexible to rigid, grayish or cinereous, rarely slightly brownish, minutely tomentose to glabrous, zonate, margin thin and acute; context whitish, floccose to corky, 0.2–1 cm. thick; tubes 0.1–1 cm. long, mouths whitish to cinereous, sometimes slightly reddish, darker when bruised, subcircular at times but usually sinuous, dædaloid, or labyrinthiform, sometimes becom-

ing lamellate in old plants, 0.5–1.5 mm. broad; spores white, smooth, oblong, mostly curved, 1.5–2 x 6.2–7.5 μ.

On dead wood or on living trees, especially of *Salix*. Common.

This is a very variable species. Sometimes very thin forms are found and such have been considered as species at different times. *Trametes rubescens* Alb. & Schw. ex Fries is a thin form with a reddish hymenium. For illustrations, see Hard, Mushrooms *f. 358.*, White, Hymen. Conn. *pl. 34. f. 2.*, and Moffatt, Higher fungi of the Chicago region *pl. 18.*

4. **D. quercina** L. ex Fries, Syst. Myc. 1: 333. 1821.

Agaricus quercinus L. Sp. Plant. 1176. 1753.

Plants annual, or sometimes reviving, sessile; pileus dimidiate, convex, 4–12 x 4–15 x 1.5–6 cm., corky, whitish to umbrinous or almost black, glabrous, margin usually thick and obtuse; context whitish, corky, 0.2–1 cm. thick; tubes 1–2 cm. long, the mouths whitish to umber, rarely circular, more often labyrinthiform and elongate or lamellate, 1 mm. or more broad, edges thick and entire.

On *Castanea* and *Quercus*, sometimes on the living trees. Rare.

This species is distinct from all of the others in its habitat, the thickness of the pileus, and the larger sinuous pores. Hard (Mushrooms *f. 357*), and White (Hymen. Conn. *pl. 34. f. 1*) give illustrations of the plant.

LENZITES Fries, Gen. Hymen. 10. 1836.

Pileus coriaceous to corky, dry and floccose in texture. Lamellæ coriaceous, firm, sometimes simple and unequal, sometimes anastomosing behind and forming pores; trama floccose and similar to the pileus, the edge subacute. Dimidiate, sessile, persistent fungi growing on wood and resembling *Dædalea*. (The above description is according to Fries, Epicr. Syst. Myc. 403.)

This genus is intermediate in position between the *Agaricaceæ* and the *Polyporaceæ* and is sometimes included among the white spored genera of the former family.

KEY TO THE SPECIES

Context white.....*1. L. betulina*
Context brown..... 1
1. Tubes or interspaces 1 mm. or more broad; lamellæ usually not much anastomosing.....*3. L. sæpiaria*
1. Tubes or interspaces less than 1 mm. broad; lamellæ freely anastomosing.....*2. L. vialis*

1. L. betulina L. ex Fries, Epicr. Syst. Myc. 405. 1838.
Agaricus betulinus L. Sp. Plant. 1176. 1753.

Plant annual, sessile, often imbricate; pileus dimidiate, 2–5 x 2–9 x 0.3–1 cm., coriaceous to somewhat corky, prevailing color grayish to brownish, marked with many narrow, multicolored zones, tomentose; margin thin and acute; context white, usually not more than 1 mm. thick; hymenium usually lamellate but sometimes poroid, the lamellæ coriaceous, about 1 mm. apart, 0.3–1 cm. broad, white or whitish; spores globose, smooth, hyaline, 5–6 μ in diameter.

On all kinds of dead wood of deciduous trees. Common.

L. flaccida Fries as reported from Ohio is but a form of this species. The plant is well represented by Hard (Mushrooms *f. 185–86*), Lloyd (Photogr. *pl. 14*), and fairly well in Cooke, Illustrations *pl. 1145*.

2. L. vialis Peck, Ann. Rept. N. Y. State Mus. **26**: 67. 1874.

Dædalea pallido-fulva Berk. Hooker's Lond. Jour. Bot. **6**: 322. 1847.

Pileus annual, sessile, imbricate or single, dimidiate or laterally connate, 1–4 x 2–7 x 0.2–0.8 cm., coriaceous or corky, grayish brown to cinnamon-brown, often darker at the base, subtomentose to glabrous, azonate or subzonate, margin acute; context brown, floccose-fibrous to soft-corky, 1–4 mm. thick; hymenium more or less poroid or labyrinthiform, rarely decidedly lamellate, pores or lamellæ averaging 2–3 to a mm., 1–3 mm. broad, concolorous with the surface of the pileus; spores cylindrical to elliptical, smooth, hyaline, 2.7–4 x 7–8.2 μ.

On dead wood of both deciduous and coniferous trees. Common.

The species was described from Ohio as *Dædalea pallido-fulva* Berk. and so reported by Morgan.

3. **L. saepiaria** Fries, Epicr. Syst. Myc. 407. 1838.
Dædalea sæpiaria Fr. Obs. Myc. 1: 105. 1815.

Plants annual, sessile, often imbricate; pileus dimidiate or reniform, 1–5 x 2–7 x 0.3–1 cm., coriaceous to corky, bright yellowish red to dark ferruginous, often lighter or discolored with age, strigose-tomentose, zonate, margin thin; context fulvous to ferruginous, floccose to soft-corky, not more than 3 mm. thick; hymenium usually lamellate, the lamellæ about 1 mm. apart, 2–5 mm. broad, rarely anastomosing, fulvous to rusty brown; spores cylindrical, smooth, white, 2.7–4 x 2–10.2 μ.

Always found on dead wood of coniferous trees. Frequent.

Easily distinguished from the preceding species by the deeper color throughout and by the more distant lamellæ that rarely anastomose.

CYCLOMYCES Kunz. & Fries, Linnæa 5: 512. 1830.

Plants annual, terrestrial and stipitate in our species, coriaceous, fuscous or cinnamon-colored; context brownish, sometimes rusty brown, floccose to fibrous; hymenium poroid at first but soon breaking up into concentric lamellæ.

The genus is distinct from all others in the concentric arrangement of the lamellæ.

1. **C. Greenei** Berk. Hooker's Lond. Jour. Bot. 4: 306. 1845.

Pileus stipitate, circular in outline, usually depressed on top, 2.5–9 cm. broad, 0.5–2 cm. thick, coriaceous when fresh, rigid when dry, yellowish brown to rusty or purplish brown, tomentose at first but becoming glabrous, more or less zonate, margin thin and acute; context fulvous to cinnamon-brown, soft floccose to fibrous or somewhat friable, thin at the margin, thicker next the stipe; tubes 5–8 mm. long, soon breaking up to form brownish concentric lamellæ; stipe central or subcentral, expanding above into the pileus, velvety, somewhat spongy, 2–7 cm. long, 0.7–2 cm. thick, fulvous to rusty brown in color.

On the ground in woods. Rare.

The species was reported from Ohio by Hard but I think has not otherwise been collected. For illustration see Hard, Mushrooms *f. 360–61.*

FAVOLUS Fries, Elench. Fung. 1: 44. 1828.

Plants annual, epixylous, more or less stipitate; pileus fleshy-tough when fresh, small or medium sized; context white, thin; tubes in a single layer, the mouths angular, usually hexagonal, often radiating outward from the stipe and somewhat longer in the radial direction; spores white.

In our species the stipe is much reduced and is usually lateral or at least eccentric. The genus is separated from *Polyporus* by the large favoloid pores, although some stipitate species of *Polyporus* closely approach in pore form the condition ascribed to this family.

KEY TO THE SPECIES

Plants about 2 cm. long and broad; hymenium more or less waxy or gelatinous.....*1. F. rhipidium*
Plants larger than above; hymenium not gelatinous or waxy.....*2. F. canadensis*

1. F. rhipidium Berk. Hooker's Lond. Jour. Bot. **6**: 319. 1847.

Plants stipitate; pileus reniform, cæspitose-imbricate, 2 cm. long and broad, coriaceous, alutaceous to white, the cuticle breaking up into minute furfuraceous squamules, concentrically sulcate; context whitish, thin; tubes short, less than 2 mm. long, more or less waxy and gelatinous, the mouths white, angular to elongate, denticulate, averaging 2–3 to a mm.; stipe lateral, pruinose, 6–7 mm. long.

On dead wood. Rare.

The above description is adapted from the original. The species was originally described from Ohio from specimens collected by Lea. Morgan also probably collected it, but otherwise it is not known from the state. In habit and color it resembles *Panus stypticus*.

2. F. canadensis Klotzsch, Linnæa **7**: 197. 1832.

F. ohiensis Berk. & Mont. Syll. Crypt. 171. 1856. *F. striatulus* Ellis & Ev. Am. Nat. **31**: 339. 1856.

Plants stipitate, the stipe often reduced to a lateral tubercle; pileus dimidiate to reniform, 1–4 x 1–8 x 0.1–0.7 cm., fleshy-tough when fresh, rigid when dry, at first reddish brown due to the presence of innate fibrils of that color, later becoming glabrous and fading to cream color or pure white, azonate,

margin thin and acute, often involute, especially on drying; context white or whitish, fleshy-tough, becoming firmer on drying, 0.5–2 mm. thick; tubes 1–5 mm. long, the mouths whitish to yellowish, distinctly angular, usually rhomboid or hexagonal, often radiating outward from the stem and longer in the radial direction, very variable in size, 0.5–3 mm. long and averaging 1–3 to a mm. in transverse direction; stipe lateral or rarely subcentral, often rudimentary, not more than 1 cm. long, 1.5–7 mm. thick.

On dead branches of deciduous trees, especially *Hicoria*. Common.

F. striatulus Ellis & Ev. is supposed to differ from *F. canadensis* in having a pileus white in color from the first, and in the smaller pores. In Ohio both of these forms are found and the writer has come to the conclusion that *F. striatulus* is to be regarded as only a form of this rather polymorphic species, for the following reasons: First, specimens of *F. canadensis* frequently become whitish in color quite early in development; second, the small pores said to be characteristic of *F. striatulus* are also frequently found in specimens with the reddish brown pileus. In attempting to separate the plants into two species one finds reddish brown specimens with either large or small pores, and white specimens with either large or small pores. The species is illustrated in Hard, Mushrooms *f. 359*.

GLOEOPORUS Mont. Hist. Cuba 385. 1838.

Plants annual, sessile or effused-reflexed; pileus small, thin and coriaceous; context fibrous, thin, usually white; tubes short, more or less gelatinous or waxy and in our species separating from the context in a thin, elastic layer when fresh or when moistened. The genus is distinct from all others in the gelatinous and at the same time separable hymenium. One species only is found in our flora.

1. **G. conchoides** Mont. Hist. Cuba *pl. 15. f. 1.* 1838.

Sessile or effused-reflexed; pileus dimidiate or conchate, 0.5–3 x 1–4 x 0.1–0.5 cm., coriaceous when fresh, rigid when dry, white or cream-colored, velvety to glabrous, azonate, margin thin, acute, with a narrow sterile band below; context white, soft-fibrous, 1–4 mm. thick; tubes less than 1 mm. long,

gelatinous or waxy and separating from the context in a thin elastic layer when fresh or when moistened, the mouths flesh-colored to reddish purple or purplish black, circular, minute, averaging 5–6 to a mm.

On dead wood of deciduous trees. Common.

The waxy separating hymenium, reddish purple in color, will serve to distinguish this species. The plant has been known as *Polyporus dichrous* Fries.

MERULIUS Haller ex Fries,
Syst. Myc. 1: 326. 1821; Haller, Hist. Stip. Helv. 3: 150. 1768.

Hymenophore formed from a mycelial mucedinous context and giving rise to shallow irregular pores formed by the intersection of obtuse folds of the hymenium; resupinate or pileate, more or less waxy in texture. Growing on rotting wood.

This genus is a very natural one and forms a transition stage from the *Polyporaceæ* to the *Thelephoraceæ* through the genus *Phlebia* of the *Hydnaceæ*. No special study of the genus has been made and only the two common species are included here, although several others have been reported from the state.

KEY TO THE SPECIES

Pileus always present, distinctly pinkish red when fresh.....*1. M. rubellus*
Pileus when present whitish or somewhat flesh-colored but not distinctly pinkish red.....*2. M. tremellosus*

1. M. rubellus Peck, Bot. Gaz. 7: 44. 1882.

Pileus sessile or effused-reflexed, dimidiate, often imbricate, 3–5 x 5–7.5 x 0.2–0.5 cm., coriaceous-cartilaginous, scarcely waxy or gelatinous, deep pinkish red, often fading with age, finely tomentose, azonate, margin thin, acute; context white or light colored, tough when fresh, soft when dry, 1–4 mm. thick; tubes short, less than 1 mm. long, formed by anastomosing veins, averaging 1–2 to a mm., cream-colored or whitish; spores (teste Peck) minute, elliptical, hyaline 4–5 x 2.5–3 μ.

On dead wood of deciduous trees. Common.

This plant is distinguished from the next one by the firmer consistency and the color, although the color of the pileus often fades in mature plants. Hard (Mushrooms *f. 353*) gives a good illustration of the plant.

2. **M. tremellosus** Schrad. ex Fries, Syst. Myc. 1: 327. 1821.

M. tremellosus Schrad. Spic. Fl. Ger. 139. 1794.

Sessile, effused-reflexed, or entirely resupinate; pileus dimidiate, 0–5 x 3–8 x 0.1–0.3 cm., fleshy or gelatinous-waxy, white or whitish, tomentose, azonate, margin thin and acute; context whitish, soft, 1–2 mm. thick; tubes very short, formed by anastomosing ridges or veins, averaging 1–2 to a mm., whitish or somewhat flesh-colored, in resupinate forms with a wide, thin, sterile border.

On old logs in woods. Common.

Quite often the plant is entirely resupinate and probably always so in young stages. The form of the hymenium is exceptionally well shown in Atkinson, Mushrooms *f. 191–92.*

Besides the above species, *M. lacrymans* Jacq. ex Fries has been included in practically every list of fungi reported from the states east of the Mississippi River, but its frequency of occurrence is probably in inverse ratio to the number of times reported. At any rate it is to be considered as a rare fungus in this country. I have never met with specimens in Ohio that I could so refer.

IRPEX Fries, Elench. Fung. 1: 142. 1828.

Hymenium inferior, dentate–lacerate from the first. Teeth concrete with the pileus, firm, subcoriaceous, acute, reticulately disposed or arranged in rows, in sessile forms connected at the base and gill-like, or favoloid in resupinate forms. Basidia 4-spored. Woody, subsessile or resupinate fungi allied to *Lenzites* and *Dædalea.* (Adapted from Fries, Hymen. Eur. 619.)

This genus is sometimes included in the *Hydnaceæ* but in at least one of the three species here described the hymenium is not toothed from the first, but is decidedly poroid and shows very close relationships to certain species of the thin pileate members of the genus *Polyporus,* e. g., *P. biformis, P. prolificans* etc., in which the hymenium soon becomes broken up into teeth. For this reason and because the plants are very common in our woods the three following species are described and most of the collections usually obtained will be found to answer to one of these descriptions.

KEY TO THE SPECIES

Context white or whitish..... 1
Context brown or brownish..... 2
1. Context less than 2 mm. thick; tubes or teeth less than 5 mm. long; pileus villous..... *1. I. tulipifera*
1. Context more than 2 mm. thick; tubes or teeth more than 5 mm. long..*2. I. mollis*
2. Hymenium cinnamon-brown.....*3. I. cinnamomeus*
2. Hymenium grayish green to olivaceous.....*4. I. farinaceus*

1. **I. tulipifera** Schw. ex Fries, Epicr. Syst. Myc. 523. 1838. *Boletus tulipifera* Schw. Syn. Fung. Car. 99. 1822.

Plants sessile, effused-reflexed, or entirely resupinate; pileus dimidiate to elongate in outline, 0–1 x 1–3 x 0.1–0.6 cm., coriaceous, white or whitish, villous, zonate, margin thin and acute; context white, fibrous, 0.5–2 mm. thick; tubes 1–5 mm. long, the mouths light colored, averaging 2 to a mm., soon breaking up into compressed teeth that are connected at the base, and often with a concentric arrangement.

On dead wood of deciduous trees. Common.

From *I. cinnamomeus* Fries, and *I. farinaceus* Fries this plant is separated by the white or whitish color, and from *I. mollis* Berk. & Curt. by the much thinner pileus and the shorter tubes or teeth.

2. **I. mollis** Berk. & Curt. Jour. Bot. & Kew Misc. 1: 236. 1849.

Pileus sessile or effused-reflexed, dimidiate, 2–5 x 5–10 x 1–3 cm., coriaceous, white or whitish, minutely tomentose to glabrous, azonate, margin thin and acute; context white, 2–6 mm. thick, fibrous; hymenium usually irpiciform, the teeth white, coriaceous, 0.5–1.5 cm. long, compressed, united at the base.

On dead wood of deciduous trees.

This plant was reported from the Miami valley by Morgan. I have not collected it in Ohio. It is much thicker than *I. tulipifera* Schw. ex Fries, and the teeth are much longer.

3. **I. cinnamomeus** Fries, Epicr. Syst. Myc. 524. 1838.

Pileus none, fungus usually entirely resupinate, coriaceous in texture, 2–5 mm. thick, entirely cinnamon-brown; context brown, not more than 1 mm. thick, fibrous; tubes or teeth 1–5 mm. long, becoming toothed at a very early stage, cinnamon-brown in color, more or less flattened, connected at the base.

On dead wood, especially of species of *Acer*. Rather common.

Distinguished from the other species here listed by the uniform brown color.

4. **I. farinaceus** Fries, Linnæa **5**: 523. 1830.

Pileus sessile, effused-reflexed, or resupinate, dimidiate, 0–0.5 x 1–3 x 0.1–0.3 cm., coriaceous, deep brown, finely tomentose, zonate, margin thin and acute; context dark brown, fibrous, less than 1 mm. thick; tubes 0.5–1.5 mm. long, mouths usually grayish green or yellowish green, averaging 2–3 to a mm., soon breaking up into teeth.

On dead wood of deciduous trees. Not common.

Sometimes the fungus is entirely resupinate and then it usually has a narrow brown margin. It is distinct from all of the other species in having a greenish hymenium.

INDEX TO THE SPECIES

Names in italics are synonyms, rejected species, etc.

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